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Part 2

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Begin the article with a short summary.

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Tables
Submit each table on a separate page, typed double spaced. Please use standard abbreviations; otherwise spell out.

References
Submit references typed double spaced. Follow the standard form of *Index Medicus:* author, title, name of journal, volume number, page or inclusive pages, and year. In book references, give author, title of book, edition (if more than one), city, name of publisher, date of book, volume number, and pages cited. References in text should begin with number 1 and continue consecutively.

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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

Proceedings of the Symposium on Quinones as Anticancer Agents

Drug Development Branch
Drug Research and Development
Division of Cancer Treatment
National Cancer Institute

Bethesda, Md
December 7, 1973

Foreword

John S. Driscoll [1]

A number of anticancer drugs of current clinical and research interest contain quinone or hydroquinone systems. On December 7, 1973, the Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, sponsored a symposium entitled "Quinones as Anticancer Agents." The objectives of this meeting were: (*a*) to describe the current research in the area of quinones as anticancer agents; (*b*) to present the structure-activity studies in this field; and (*c*) to discuss on an interdisciplinary basis the data obtained by chemists, physicians, and pharmacologists in order to evaluate ongoing studies and to determine whether additional investigations might be appropriate. Summaries of the formal presentations follow. The purpose of this publication is to present the work of some of the active investigators in the field so that interested scientists might have contact points for further discussions.

[1] Drug Design and Chemistry Section, Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

Quinone Structure-Antitumor Activity Relationships

John S. Driscoll [1,2]

The Drug Research and Development (DR&D) effort at the National Cancer Institute (NCI) is concerned with obtaining new antitumor drug candidates and making the initial evaluation of these materials in animal models to determine their potential as useful antitumor agents. As a result of approximately 17 years of effort, DR&D now has data available on more than 200,000 synthetic compounds.

Because of a general policy to disseminate this information as widely as possible, structure-activity studies are carried out by the NCI on classes of compounds with recognized antitumor properties. Nucleoside (1), folic acid (2), and methanesulfonate (3) derivatives have been treated previously. It was felt that a similar study of the 1500 quinones tested by the NCI would be useful to workers in cancer chemotherapy, and this recently completed project (4) had the following general objectives:

1. The organization of quinone structural and antitumor data into a useful format;
2. Analysis of the data in order to study possible structure-activity relationships.

Compounds were organized into 12 structural groups (table 1). Within each of these groups, the compounds were further organized into subgroups to allow structure searches.

The second type of data organization utilized was the Compound Family. Certain groups of compounds of special interest in cancer chemotherapy were organized into families based on common structural features. Parent structures for some of these families are shown in figure 1.

TABLE 1.—Quinone structural groups

Group	Structural type
1	Benzoquinones
2	Naphthoquinones
3	Anthraquinones
4	Benzanthraquinones
5	Polyaromatic quinones
6	Bridged bicyclic quinones
7	*N*-Heterocyclic quinones
8	*O*-Heterocyclic quinones
9	*S* and multiple hetero-atom quinones
10	Bianthrones
11	*o*-Quinones
12	Partially reduced or oxidized quinones

Biologic activity was summarized for four in vivo test systems (L1210 leukemia, Walker 256 carcinosarcoma, Adenocarcinoma 755, and Sarcoma 180) and one cell culture assay (KB).

Structure-activity data were collected into tables and analyzed in three separate ways—by Structural Group, by Compound Family, and by Biologic Activity. A summary of these data was presented (4).

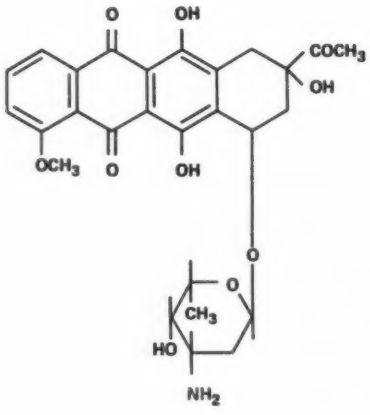
Several general conclusions can be made concerning quinone antitumor activity:

1. Quinone natural products make up a large proportion of the active compounds (daunorubicin, adriamycin, mitomycin C, streptonigrin, lapachol).[3]
2. Considering both in vivo and in vitro activity for the four structural groups with more than 150 members, the following percentage activity occurs:

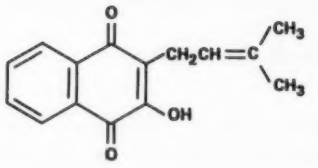
[1] Drug Design and Chemistry Section, Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

[2] *Reprint requests to:* John S. Driscoll, PhD, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bldg 37, Rm 6D24, Bethesda, Md 20014.

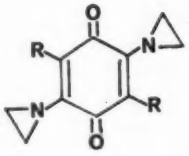
[3] Daunorubicin: NSC-83142; CAS reg. No. 23541-50-6; daunomycin (NSC-82151).
Adriamycin: NSC-123127.
Mitomycin C: NSC-26980; CAS reg. No. 50-07-7.
Streptonigrin: NSC-45383; CAS reg. No. 632-62-2.
Lapachol: NSC-11905; 1,4-naphthoquinone, 2-hydroxy-3-(3-methyl-2-butenyl)-.



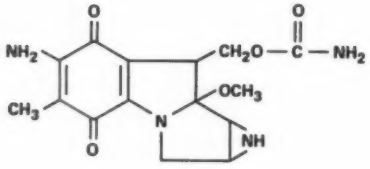
DAUNORUBICIN



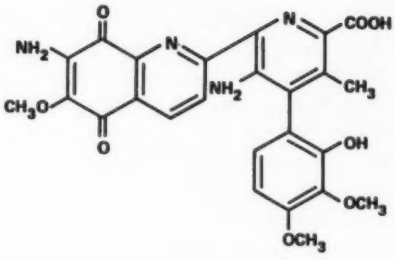
LAPACHOL



AZIRIDINYL QUINONES



MITOMYCIN C



STREPTONIGRIN

FIGURE 1.—Parent structures for some of the quinone families.

Group	*No. of actives/No. tested*
Benzoquinones	30/266 (11%)
Naphthoquinones	30/458 (7%)
Anthraquinones	9/379 (2%)
N-Heterocyclic quinones	24/151 (16%)

3. The most interesting family of synthetic compounds is the aziridinyl benzoquinones which have central nervous system antitumor activity.

4. There appears to be a correlation between KB in vitro cytotoxicity and L1210 in vivo activity among the quinones in this study.

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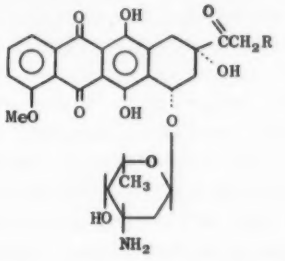
Adriamycin (NSC-123127) and its Analogs

David W. Henry [1,2,3]

Adriamycin (*1*) is probably the most prominent quinone-related antitumor drug in use. It is a product of a mutant strain of *Streptomyces peucetius*, the fungal species that produces the closely related drug, daunorubicin (*2*). These compounds are members of the large class of anthracycline antibiotics, many members of which have growth-inhibiting properties. Daunorubicin was used clinically in the treatment of acute granulocytic and lymphocytic leukemia before the advent of adriamycin, but the latter appears to be active against a much broader spectrum of tumors, especially solid tumors, and is currently under intense clinical investigation. Table 1 presents overall response rates of some human tumors to adriamycin treatment (1).



1 R = OH adriamycin
2 R = H daunorubicin

Both antibiotics display side effects common to many antitumor drugs (eg, bone marrow depression, stomatitis, alopecia), but their dose-limiting side effect is primarily cardiotoxicity. Both drugs are thought to act by inhibition of cellular nucleic acid metabolism, but adriamycin appears therapeutically advantageous because of more optimal uptake properties (2) or because of possible decreased host immunosuppression (3). At the molecular level, adriamycin and daunorubicin appear to be very similar, and it is very encouraging that the small structural difference between them can so favorably affect therapeutic characteristics. Thus, there is justification for the hope that modifications of the parent drugs can result in greater effectiveness and perhaps decreased toxicity.

TABLE 1.—Overall response rates of some human tumors to adriamycin*

Tumor	Response rate (%)
Sarcoma	26
Breast cancer	36
Bronchogenic carcinoma	19
Malignant lymphoma	41
Neuroblastoma	36
Acute leukemia	24

*Data from ref 1.

Based on these considerations and on published information on structure-activity relationships, Stanford Research Institute is pursuing four approaches to developing the adriamycin-daunorubicin lead area: (*a*) total synthesis of adriamycin, (*b*) synthesis of derivatives of the parent antibiotics, (*c*) semisynthetic analogs, (*d*) totally synthetic analogs.

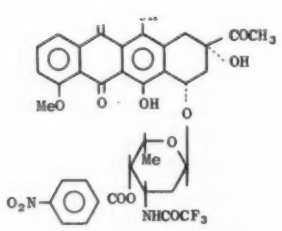
Total synthesis is included because adriamycin is an exceptionally expensive drug and the experience gained in such a synthesis should be very valuable for the preparation of analogs. We have recently succeeded in coupling

[1] Department of Bio-Organic Chemistry, Stanford Research Institute, Menlo Park, Calif.

[2] The author gratefully acknowledges the contributions of E. M. Acton, A. N. Fujiwara, R. B. Bicknell, and K. Yamamoto, who were responsible for the synthesis of analogs 6-8, and D. R. Black and D. L. Taylor, who performed the nucleic acid inhibition studies.

[3] *Reprint requests to:* David W. Henry, PhD, Department of Bio-Organic Chemistry, Stanford Research Institute, Menlo Park, Calif 94025.

[4] Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.



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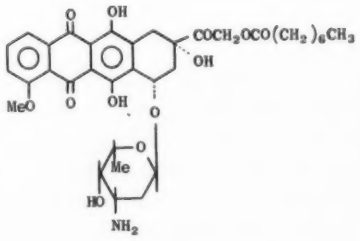

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a blocked daunosamine derivative with daunomycinone to obtain compound *3*; subsequent deblocking gave daunorubicin (4). This sequence formally completes the total synthesis of adriamycin when coupled with the total synthesis of daunosamine by Marsh et al (5), the conversion of daunorubicin to adriamycin by Arcamone and co-workers (6), and the recent synthesis of daunomycinone by Wong et al (7). The synthesis of the aglycone, adriamycinone, via Diels-Alder reactions of anthracene-1,4, 9,10-diquinone derivatives, is being actively explored (8).

Table 2 lists assay data for several daunorubicin derivatives (9,10) that are as effective as the parent in at least one test system. In every case, drug potency is reduced but toxicity is also decreased. The response to various dosage regimens is also altered by derivatization. In general, the ketone derivatives retain more potency than the amine derivatives. In other experimental test systems, some derivatives provide excellent properties. The glycoloylhydrazone (NSC-146496) is partially curative against P388 leukemia and B16 melanoma and the *N*-acetyl derivative (NSC-118714) is also very effective in the P388 test. The benzhydrazone (rubidazone, NSC-164011) was developed in France (10) and has shown highly significant results in all four of the signal mouse tumors used by the National Cancer Institute for primary drug evaluation (L1210 leukemia, P388 leukemia, B16 melanoma, and Lewis lung carcinoma). Favorable clinical results against leukemia have been reported for rubidazone (10). Derivative work on adriamycin has been more limited, but Arcamone et al (12) have found that 14-*O*-acyl derivatives (eg, compound *4*) may have improved activity in some animal tumor models (12).

The possibility that daunorubicin would display improved antitumor effects when administered as a complex with DNA was discerned by Trouet and subsequently found to be correct in the treatment of human leukemia (13). We have found that the therapeutic gain provided by this technique extends to the adriamycin-DNA complex in animal models. Table 3 lists comparative data on the free drugs and their DNA complexes. The first two pairs of entries in the table compare equal antibiotic dosages, administered as free drug and as complex. The last two entries demonstrate the reduced toxicity of the daunorubicin-DNA complex when compared to the free drug.

Because of the favorable effects achieved by the various types of derivatization of the parent antibiotics, we are exploring additional compounds related to rubidazone and attempting to extend to derivatives the therapeutic gains of DNA complexation.

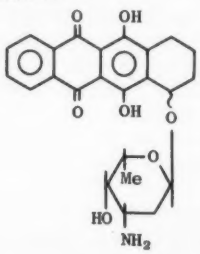

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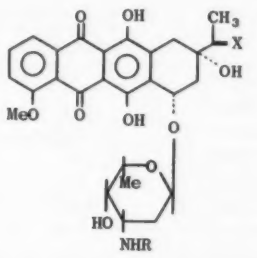
TABLE 2.—Assay data for selected daunorubicin derivatives



NSC No.	R	X	Inhibition (ED50) of DNA synthesis* (μmol)	T/C† qd, Days 1,5,9 (mg/kg)	T/C† qd, Days 1–9 (mg/kg)
143114	H	=NNHCONH$_2$	18	157 (50) Toxic (100)	150 (6.3) Toxic (50)
164011	H	=NNHCOC$_6$H$_5$	2.7	205 (16) (1/6 were cured)	127 (5.9)
146496	H	=NNHCOCH$_2$OH	3.5	139 (21)	142 (7.0)
143673	CSNHC$_6$H$_5$	0	8.1	131 (75)	138 (25)
118714	COCH$_3$	0	7.5	126 (40)	126 (20)
83142	Daunorubicin		0.3	144 (1.8) Toxic (16)	125 (2.0) Toxic (8)
123127	Adriamycin		0.8	155 (5.0)	130 (2.0)
180024	Carminomycin		0.06	—	—

*L1210 cells in culture.

†From National Cancer Institute L1210 antitumor assays (ref 11). T/C = ratio (× 100) of the survival time of treated mice to that of untreated controls.

Semisynthetic derivatives of adriamycin and daunorubicin contain either a natural aglycone or daunosamine as an element of their structure. Coupling products of daunomycinone with glucose and glucosamine have been prepared (14), but significant biologic activity in these compounds has not been reported. Using the technique devised for coupling daunosamine to daunomycinone, both diastereomers of semisynthetic analog ***5*** were prepared (15). Interestingly, the natural (S) and unnatural (R) diastereomers were equally active as inhibitors of DNA synthesis in cultured L1210 cells, providing ED50 values of 5.6 and 5.4 μmol respectively. The comparable value for adriamycin is 0.8 μmol. In vivo antitumor tests on these compounds are pending. Work on a variety of semisynthetic analogs containing both fraudulent aglycones and amino sugars is in progress. Compounds with aromatic aglycones at various levels of oxidation are being synthesized in an attempt to determine whether the quinone character of the natural products is functional or incidental.

Among totally synthetic analogs, compounds ***6–8*** are of interest. In vivo L1210 antitumor activity was not seen with ***6*** or ***7***; assays on *8* are pending. However, all compounds inhibit

TABLE 3.—Effect of DNA complexes of adriamycin and daunorubicin on survival time of mice bearing L1210 ascites tumors*

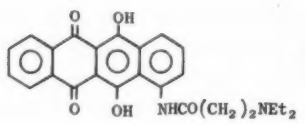
NSC No.	Drug	Dose (mg/kg)	T/C	No. of cures/ No. treated
123127	Adriamycin	5.0	155	0/10
169534	Adriamycin-DNA	50†	194	2/10
83142	Daunorubicin	1.8	144	0/10
169533	Daunorubicin-DNA	17†	155	0/10
83142	Daunorubicin	8.3	111	0/10
169533	Daunorubicin-DNA	80†	161	0/10

*From National Cancer Institute studies (ref 11); qd, Days 1,5,9, ip.

†The parent antibiotic constitutes 10% of the weight of the complex.

DNA synthesis in cultured L1210 cells at significant levels. The ED50 values for *6*–*8* are 49, 2.6, and 8.2 μmol respectively. Additional synthetic work on congeners of *7* and *8* is being actively pursued.

The derivative and analog work has had valuable guidance from molecular model studies of possible drug-binding modes to helical DNA. Based on the crystallographically established conformation of *N*-bromoacetyl daunorubicin (16), it is possible to propose a single, most probable, drug-DNA complex that embraces the known intercalative binding mode of the drug (17). This binding scheme allows the aglycone chromophore to be largely overlapped by the enclosing base pairs while the daunosamine side chain projects into the major groove with its ammonium group closely adjacent to a phosphate of the deoxyribosephosphate backbone. The tertiary hydroxy of the aglycone and the secondary hydroxy of the sugar are close to other phosphate groups and may form hydrogen bonds. This model is qualitatively consistent with most biochemical and structure-activity data developed from derivatives and analogs in the area. It rationalizes the retained activity of C-14 carbonyl derivatives (no steric interference with binding at this position) and the reduced or destroyed activity of *N*-acylated derivatives (reduced binding due to loss of the positive charge of the ammonium ion). The pronounced inhibition of DNA synthesis displayed by *7* and *8* (totally synthetic compounds that also fit the receptor site) also supports the hypothesis. One result that clearly contradicts this receptor site concept, and which is being investigated further through current structure-activity studies, is the equal DNA synthesis-inhibiting properties of the two diastereomers of compound *5*. The isomer of unnatural configuration has a shape that cannot fit the proposed receptor site, thus suggesting that inhibition of nucleic acid synthesis through binding to DNA may not be the mechanism, or the only mechanism, by which these compounds inhibit growth.



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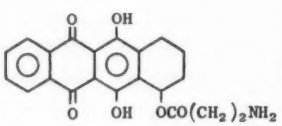

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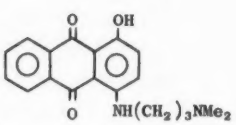

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LIST OF COMPOUNDS

Adriamycin: NSC-123127

Carminomycin: NSC-180024; 5,12-naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoxyl)oxy]-7,8,9,10-tetrahydro-1,6,8,11-tetrahydroxy-, (8*S*-cis)-

Daunorubicin: NSC-83142; CAS reg. No. 23541-50-6; daunomycin (NSC-82151)

NSC-118714: daunomycin, *N*-acetyl-, compd. with isopropyl alcohol (1:1)

NSC-143114: daunomycin, 3-semicarbazone, monohydrochloride, dihydrate

NSC-143673: daunomycin, 3-(3-phenyl-2-thioureido)-, monohydrate

NSC-146496: daunomycin, glycoloylhydrazone, monohydrochloride, monohydrate

NSC-169533: nucleic acid, deoxyribo-, sodium salt, compd. with (8*S-cis*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride

NSC-169534: nucleic acid, deoxyribo-, sodium salt, compd. with (8*S-cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride

Rubidazone: NSC-164011

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Quinone Natural Products: Streptonigrin (NSC-45383) and Lapachol (NSC-11905) Structure-Activity Relationships [1,2]

Koppaka V. Rao [3,4]

Streptonigrin

Streptonigrin is an antitumor antibiotic produced by *Streptomyces flocculus* (1). Bruneomycin,[5] isolated recently in the USSR, is identical to streptonigrin (2). The compound shows antitumor activity in a wide range of experimental tumors. Moderate to high activity is shown against transplanted tumors such as Sarcoma 180, Adenocarcinoma 755, Lewis lung carcinoma, Ridgway osteogenic sarcoma, Walker 256 (W256) carcinosarcoma, and a spontaneous tumor such as mouse mammary carcinoma (3,4). The human tumor HS No. 1 grown in rats is particularly sensitive (3,5). Also, several viral tumors such as Rauscher and Friend leukemia are significantly inhibited by streptonigrin (6,7).

Streptonigrin is involved in DNA metabolism, although only fragmentary details on its exact role are available (8–12). In clinical trials, the drug produces significant objective responses in certain forms of lymphoma (13–15). In a double-blind test, its activity compared favorably with that of chlorambucil,[6] although streptonigrin was somewhat more toxic (16,17).

The structure of streptonigrin (***S-1***) was elucidated in 1963 (18). Its carbon skeleton is unique and several other features such as the aminoquinolinequinone system are unusual. The compound has not yet been synthesized although the synthesis of some simpler tricyclic and tetracyclic analogs has been reported (19–26).

In a brief study we developed some structure-activity relationships which will be summarized here. In ring A, the aminoquinone system was found to be essential. Replacement of the amine function by OH (***S-2a***) or OCH_3 (***S-2b***) led to loss of activity. Similarly, reductive acetylation ***(S-3)*** or reductive methylation ***(S-4)*** or conversion of compound ***S-4*** to compound ***S-5*** with a new quinone system gave inactive compounds. The monoxime (***S-6***) of streptonigrin was weakly active, but after reduction and reoxidation it gave the azastreptonigrin (NSC-76880) (***S-7***) which had activity comparable to that of streptonigrin. Isopropylideneaza-streptonigrin [7] ***(S-8)*** was also active but clinical trials produced no objective responses.

Ring B could be selectively hydrogenated to a tetrahydro derivative (***S-9***) which was inactive. In ring C, acetylation of the amine function produced an inactive compound (***S-10***). However, esterification of the carboxyl with a series of alcohols gave esters which showed significant activity. Acetylation (***S-12***) or methylation (***S-13***) of the phenolic hydroxyl of ring D gave retention of activity while complete demethylation of ring D (and A) gave compound ***S-14*** which was inactive.

In a series of substituted quinolinequinones,

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[1] The work described in this review (except for the section on the synthesis of destrioxyphenyl streptonigrin) was performed at the John L. Smith Memorial for Cancer Research (Pfizer Inc., Maywood, NJ) under the participatory supervision of the author. Contributions of associates, Mr. Herman Rutner, Dr. Nathan Lewin, and Miss Jean Yeauh, in the synthesis of several of the analogs of streptonigrin and lapachol are gratefully acknowledged. Lapachol compounds ***L-3*** and ***L-4*** were synthesized by Mr. Rutner during the second phase of the program on structure and activity of lapachol and analogs.

[2] Lapachol: CAS reg. No. 84-79-7; 1,4-napthoquinone, 2-hydroxy-3-(3-methyl-2-butenyl)-.

Streptonigrin: CAS reg. No. 632-62-2.

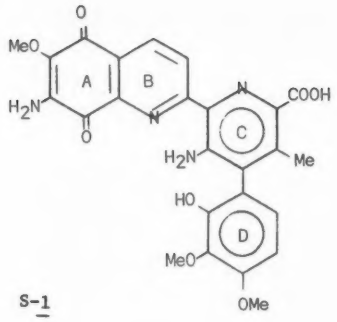
[3] J. Hillis Miller Health Center, University of Florida College of Pharmacy, Gainesville.

[4] *Reprint requests to:* Koppaka V. Rao, Professor, J. Hillis Miller Health Center, University of Florida College of Pharmacy, Box 779, Gainesville, Fla 32610.

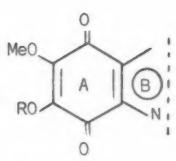
[5] Bruneomycin: CAS reg. No. 3930-19-6.

[6] Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-.

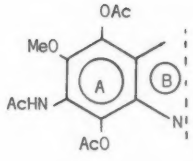
[7] Isopropylideneaza-streptonigrin: NSC-62709; CAS reg. No. 15964-31-5.



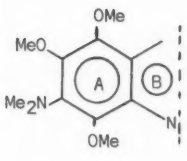
S-1



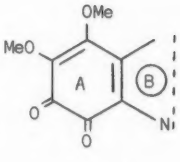
S-2a, R=H
S-2b, R=CH_3



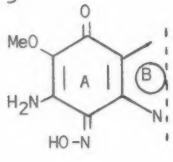
S-3



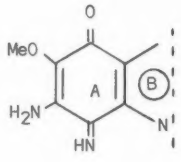
S-4



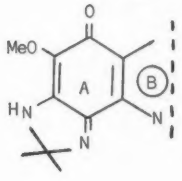
S-5



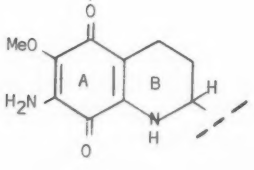
S-6



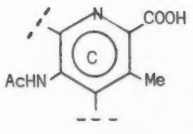
S-7



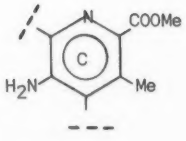
S-8



S-9



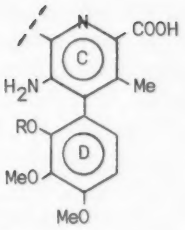
S-10



S-11

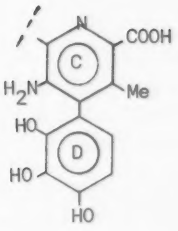
only the 6-methoxy-7-amino derivative (***S-15***) was active in an in vitro system while the isomeric quinone (***S-16***) was inactive.

On the basis of these observations, we proposed a hypothesis which defined the structural elements essential for activity; these elements are embodied in compound ***S-17***. Synthesis of a tricyclic analog such as ***S-18*** was undertaken in order to test this hypothesis. The synthesis was approached by a modified Friedlander-type condensation between a substituted nitrobenzaldehyde (to represent ring A) and a substituted 2-acetylpyridine (ring C) followed by transformations to form the aminoquinone

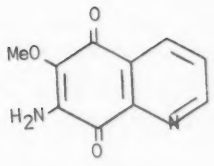


S-12, R=CH_3CO

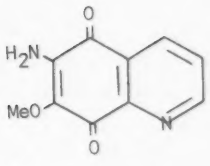
S-13, R=CH_3



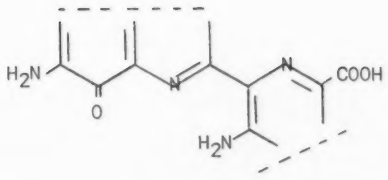
S-14



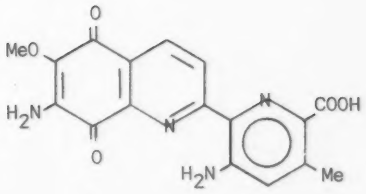
S-15



S-16



S-17



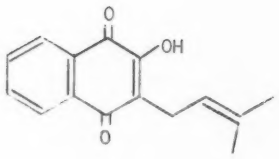
S-18

system. Preliminary results on the synthetic analog (***S-18***) indicate that it is active against *Bacillus subtilis* to a degree comparable to that of streptonigrin. Antitumor evaluation of ***S-18*** will be carried out shortly.

Lapachol

Isolation of lapachol and elucidation of its structure (***L-1***) were described during the late 19th century (28–30). Two syntheses of lapachol have also been reported (31,32), and several analogs of the drug have been found to have antimalarial activity (33).

The antitumor activity of lapachol was recognized recently (34). Extensive evaluation showed that the compound was active mainly against W256 carcinosarcoma. It was most active when given by the oral route with a



L-1

relatively high therapeutic index of nearly 20. Recent studies showed that it was active against Yoshida sarcoma and that it had antibiotic and antischistosomal activity (35,36). Lapachol was subjected to brief clinical trials (Medrek TJ, personal communication) which were discontinued because the drug produced insufficient plasma levels. The primary toxic effects were moderate to severe anemia and increased clotting time (37).

Dihydrolapachol [8] was found to be a potent inhibitor of the electron transport system (38). Howland (39) distinguished between the metabolic activities of naphthoquinones with saturated and unsaturated side chains in oxidative phosphorylation.

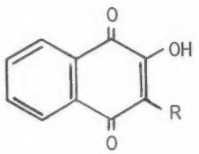
The activity of lapachol and the problems with plasma concentration prompted studies on structural variation. Changes were introduced at four points: the hydroxyl group, the quinone system, the side chain, and the carbon skeleton.

Modification of the hydroxyl group was effected by conversion to the acetate (38), the methyl ether (40), the carboxymethyl ether, or the carbethoxymethyl ether. Other changes included replacement of the hydroxyl group by amino, methylamino, hydroxyethylamino, or chlorine (with $-CH_2CH_2C(Cl)-CH_3)_2$ side chain) groups. Only the acetate was active because of its ready hydrolysis to compound ***L-1***. Similarly, several cyclic ethers such as α-lapachone (30), dehydro-α-lapachone (41), β-lapachone (30), its bromo and sulfonate derivatives (30), and α- and β-dunniones (42) were tested but were inactive.[9]

[8] Dihydrolapachol: NSC-26695; CAS reg. No. 3343-38-2; 1,4-naphthoquinone, 2-hydroxy-3-isopentyl-.

[9] α-Lapachone: NSC-26327; CAS reg. No. 4707-33-9; 2*H*-naphtho[2,3-*b*]pyran-5,10-dione, 3,4-dihydro-2,2-dimethyl-.

Dehydro-α-lapachone: NSC-106453; CAS reg. No.



R=

$CH_2CH_2CH(CH_3)_2$	(ref 44)	$CH=C(CH_3)_2$	(ref 47)
$CH_2CH_2C(Cl)(CH_3)_2$	(ref 45)	$CH=CHCH(CH_3)_2$	(ref 45)
$CH_2CH_2C(OH)(CH_3)_2$	(ref 30)	$CH=CH(CH_2)_4CH_3$	(ref 44)
$CH_2CH(OH)C(OH)(CH_3)_2$	(ref 41)	$CH=CHC_6H_5$	(ref 44)
$CH_2-C_6H_5$	(ref 46)	$CH_2-CH=CHCH_3$	(ref 31)
$CH(OH)CCl_3$		$CH_2-CH=CH_2$	(ref 46)
$CH=CH-CH_3$	(ref 44)	$C(CH_3)_2CH=CH_2$	(ref 48)
$CH=CH-CH_2CH_3$	(ref 44)	$CH_2-CH=C-CH_3$ $\quad\quad\quad

Conversion of the quinone system to 1-monoxime (43), its *O*-carboxymethyl derivative, the 1-semicarbazone, and the 1-thiosemicarbazone gave inactive products. Triacetyldihydro (30) and tripropionyldihydro derivatives prepared by reductive acylation were active, presumably because of their easy conversion to compound ***L-1.***

A series of structural changes were introduced in the side chain and these are shown in figure 1. Even though the changes were slight, none of the analogs were active except the 4′-hydroxy derivative (lomatiol)[10] which was weakly active.

In the last group, several substituted benzoquinones and two naphthoquinone derivatives (fig 2) were examined but none showed activity.

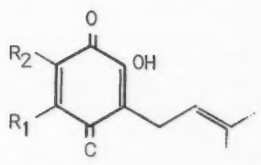
These essentially negative results indicated that the molecular structure of lapachol had a unique specificity for the biologic activity. At this time, one additional analog in which the side chain was replaced by a geranyl unit showed significant activity, lending further support to the essential nature of the isoprenoid side chain. At the same time, three urinary metabolites of lapachol were isolated in experiments with dogs; these were lomatiol, its glucuronide, and the carboxylic acid (***L-2.***) These observations and the recent findings concerning the important role of alkyl-substituted quinones in oxidative phosphorylation (49) suggested that further alterations of the side chain might be profitable.

Accordingly, with the objective of preventing oxidative degradation, two analogs were prepared in which the methyl groups were replaced by chlorine and bromine respectively (***L-3*** and ***L-4***). Both analogs showed highly significant activity in the W256 system and gave higher blood levels than the parent compound. The dichloro derivative (***L-3***) is awaiting clinical trials.

15297-92-4; 2*H*-naphtho[2,3-*b*]pyran-5,10-dione, 2,2-dimethyl-.

β-Lapachone: NSC-26326; CAS reg. No. 4707-32-8; 2*H*-naphtho[1,2-*b*]pyran-5,6-dione, 3,4-dihydro-2,2-dimethyl-.

[10] Lomatiol: NSC-24872; CAS reg. No. 523-34-2; 1,4-naphthoquinone, 2-hydroxy-3-(4-hydroxy-3-methyl-2-butenyl)-.

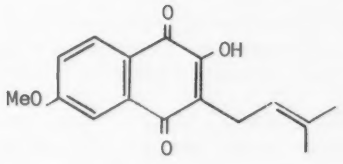


R_1 =OH, R_2 =H

R_1 =OH, R_2 =CH_2 -CH=$C(CH_3)_2$

R_1 =CH_3, R_2 =H

R_1 =C_6H_5, R_2 =H



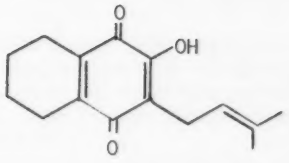
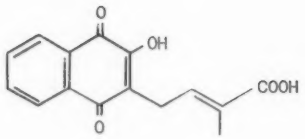
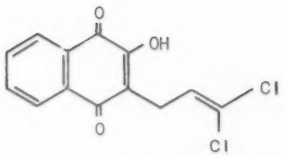


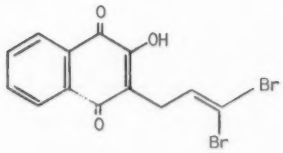
FIGURE 2.—Structures of several substituted benzoquinones and 2 naphthoquinone derivatives of lapachol.



L-2



L-3



L-4

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The Potential of Coenzyme Q_{10} (NSC-140865) in Cancer Treatment [1]

Karl Folkers [2,3]

Coenzyme Q_{10} (fig 1), like folic acid, occurs naturally in human tissue, and the specific biochemical function of each is as a vitamin. Antimetabolites of folic acid, including methotrexate,[4] have been developed into clinically useful anticancer agents. It was obvious several years ago that antimetabolites of coenzyme Q_{10} might also be developed into clinically useful anticancer agents. Indeed, many analogs of coenzyme Q_{10} were synthesized, and some were found to be highly effective inhibitors of coenzyme Q_{10}-enzyme systems in vitro. Some of these analogs showed antitumor activity in animal test systems. Consequently, the development of coenzyme Q_{10} antimetabolites became a new approach in cancer chemotherapy as based on the fundamental bioenergetics of the cell. This new approach is believed to be worthy of substantial scientific effort.

In addition to the potential utilization of antimetabolites of coenzyme Q_{10} in cancer chemotherapy, it became evident that the role of coenzyme Q_{10} in the bioenergetics of cell respiration provided a rationale for the direct administration of coenzyme Q_{10} to certain cancer patients to increase possibly the biosynthesis of antibodies in the lymphocytes. Since antibody biosynthesis in the lymphocytes is dependent upon bioenergetics, it is evident that any deficiency of coenzyme Q_{10} in the cell could impair the mitochondrial electron transfer processes and coupled oxidative phosphorylation and, thereby, impair antibody biosynthesis.

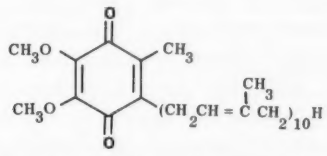

FIGURE 1.—Structure of coenzyme Q_{10}.

As a quinone, coenzyme Q_{10} of human tissue provides a unique new approach to cancer chemotherapy. Background information and exploratory data towards this goal are briefly summarized in the following sections.

Biochemical and Nutritional Aspects of Coenzyme Q_{10}

A symbolic positioning of coenzyme Q_{10} in the respiratory chain was depicted by Lehninger in his book on biochemistry published in 1970. The exact positioning of coenzyme Q_{10} in the respiratory chain is not as important to the concept of the utilization of antimetabolites of coenzyme Q_{10} in cancer chemotherapy as is the knowledge of the existence and essentiality of coenzyme Q_{10} in the chain. The complexity of the supramolecular chain foretells the difficulty of proving the exact positioning of coenzyme Q_{10} in the chain.

The mechanism of biosynthesis of coenzyme Q_{10} was initially worked out in 1966. Space does not permit citation of previous and subsequent events.

It is important to an understanding of the potentiality of antimetabolites of coenzyme Q_{10} in cancer chemotherapy to know that there are three quinone precursors of coenzyme Q_{10} which imply three enzyme sites for inhibition of biosynthesis. In addition, there are three enzyme sites in mitochondria for inhibition of the functionality of coenzyme Q_{10}. There are also an unknown number of enzyme sites of coenzyme Q_{10} in the Golgi apparatus for additional inhibitions. Therefore, there are multiple sites for inhibition of coenzyme Q_{10}-enzymes by analogs of coenzyme Q_{10}.

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[1] Coenzyme Q_{10}: CAS reg. No. 303-98-0; *p*-benzoquinone, 2-(3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl)-5,6-dimethoxy-3-methyl-; coenzyme Q-183.

[2] Institute for Biomedical Research, The University of Texas at Austin.

[3] *Reprint requests to:* Dr. Karl Folkers, Institute for Biomedical Research, The University of Texas at Austin, Austin, Tex 78712.

[4] Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)-methyl]methylamino]benzoyl]-.

During 1964–1972, the vitamin activity of coenzyme Q_{10} was repeatedly and extensively demonstrated in nutritional syndromes of the rabbit, monkey, rat, chick, turkey, and hamster. At the clinical level, at least three structural forms of coenzyme Q have been administered to human patients with certain disease conditions; the results were generally concluded to be therapeutically beneficial. Of importance, side effects or toxicity associated with the administration of coenzyme Q to man did not constitute a limitation since none were observed.

Bases for the existence of human deficiencies of coenzyme Q_{10} have been studied, and three mechanisms in human nutrition and metabolism have been recognized which can bring about deficiencies of coenzyme Q_{10} in man. Certain of these mechanisms could lead to impairment in the biosynthesis of antibodies, particularly in the lymphocytes, and could cause a predisposing unfavorable situation for resistance to cancer. These three mechanisms are as follows:

1. Nutritional deficiencies in dietary tyrosine, phenylalanine, or any of the known vitamins and minerals required for the biosynthesis could cause a deficiency of coenzyme Q_{10} in human tissue.
2. Dietary and metabolic lipid peroxidation in man can (*a*) irreversibly destroy coenzyme Q_{10} by its participation as a nonspecific antioxidant and (*b*) cause irreversible epoxidation of coenzyme Q_{10}; both reactions could cause a deficiency of coenzyme Q_{10} for vital electron transfer. A combination of nutritional deficiencies and lipid peroxidation (mechanism 1) could be a potential cause of wide-spread deficiency of coenzyme Q_{10} in man.
3. A genetic defect or inborn error of metabolism at any step of the complex biosynthesis could cause a deficiency of coenzyme Q_{10} in human tissue.

Search for Human Deficiencies of Coenzyme Q_{10}

An assay for the detection and measurement of human deficiencies of coenzyme Q_{10} has been devised on the basis of the "Brin principle," which was defined by Folkers (1) as follows:

> "The specific activity of a coenzyme-apoenzyme system is differentially assayed in the absence and in the presence of added coenzyme. A significant increase in the specific activity of the enzyme system in the presence of added coenzyme measures a deficiency of the coenzyme at the site or of the vitamin in the tissue."

The assay for deficiencies of coenzyme Q_{10} in human tissue or cells is based on the activity of the succinate dehydrogenase-coenzyme Q_{10} reductase which is differentially assayed in the absence and presence of added coenzyme Q_3. A significant increase in the specific activity of this enzyme indicates a deficiency of coenzyme Q_{10} not only at this enzyme site but in the tissue.By using this new enzyme methodology as described by Nakamura, Littarru, and Folkers (2), evidence has been obtained for the existence of deficiencies of coenzyme Q_{10} in human cardiac, gingival, and dystrophic tissues.

It is evident that deficiencies of coenzyme Q_{10} can also exist in cancer patients and can be found and measured. It seems plausible that correction of a long-standing and generalized deficiency of coenzyme Q_{10} in cancer patients would be beneficial.

Antitumor Activity of Coenzyme Q Analogs

An antimetabolite of a vitamin can be clinically used for brief periods on the basis of selectivity.

Since antimetabolites of coenzyme Q, like those of folic acid such as methotrexate, could be valuable in cancer chemotherapy, many analogs were synthesized. The exploratory testing in animal tumor systems was done in cooperation with Dr. Jean Scholler, first at the Stanford Research Institute in California and then at the University of Texas at Austin.

Data on the inhibition of Sarcoma 180 ascites tumor in mice for one such analog are shown in table 1. Results of the initial testing of this analog for inhibition of L1210 leukemia in mice are also shown in this table.

Such exemplary data on one inhibitor of the many which were synthesized and tested establish antimetabolites of coenzyme Q as a new lead in cancer chemotherapy. Antitumor activity without significant toxicity is quite possible, particularly on the basis of such selectivity in the experimental chemotherapy of malaria by

antimetabolites of coenzyme Q_{10} as reported by Wan, Porter, and Folkers (3).

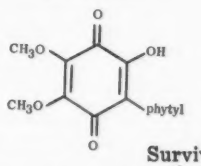
Possible Influence of Coenzyme Q_{10} on Antibody Biosynthesis

Since biosynthesis in human tissue is also dependent upon the mechanisms of bioenergetics, the rationale is apparent that cancer patients who happen to be deficient in coenzyme Q_{10} might have an impairment of antibody biosynthesis which correlates to some degree with the influence of the deficiency on bioenergetics. It is conceivable that, in turn, such impairment might be unfavorable for the immune mechanisms and resistance to cancer. Lymphocyte preparations were made from 10-ml blood samples from three advanced cancer patients and, indeed, there was a deficiency in the activity of the succinate dehydrogenase-coenzyme Q reductase. Current unpublished data by Toru Yamagami and Yoshifumi Iwamoto are shown in table 2.

Deficiencies of about 50% in coenzyme Q_{10}-enzyme activity are considered quite meaningful and indicate that the three cancer patients shown in table 2, who were in an advanced condition, might have had a depressed antibody potentiality. Such an implication is uncertain but surely indicates the need for further biomedical and clinical research on the direct use of coenzyme Q_{10} in the treatment of cancer patients to see if the immune mechanism can be benefited through improving antibody biosynthesis. For this use, coenzyme Q_{10} would not be an antitumor agent just as folic acid is not an antitumor agent. Coenzyme Q_{10} would be used for its intrinsic vitamin functionalities just as

TABLE 1.—Inhibition of Sarcoma 180 and L1210 leukemia by an analog of coenzyme Q

Compound



Test system	Dose (mg/kg/day)	No. of deaths/ No. treated	Survival time (avg days)	No. with no ascites/ No. treated	T/C
Sarcoma 180	12.5	0/10	—	6/10	0.19†
L1210 leukemia	25	—	11.0	—	125
	12.5	—	9.7	—	110
Controls*	8.8	—	—	—	—

*Control animals received 10 ml/kg/day of Tween-80 + saline.
†Total packed cell volume.

TABLE 2.—Activity of the succinate dehydrogenase coenzyme Q reductase in human lymphocytes

Patient No.	Age (yrs)	Sex	Diagnosis	SA*	SA with coenzyme Q_3	Activation coefficient	Deficiency (%)
1	60	M	Advanced kidney carcinoma	5.21	11.11	113	53
2	55	F	Advanced oral cancer	3.29	7.30	121	55
3	49	M	Advanced lung carcinoma	2.43	5.02	106	51

*SA = specific activity.

folic acid is used for its hematologic activity. On the other hand, an antimetabolite of coenzyme Q_{10} can have antitumor activity just as methotrexate as an antimetabolite of folic acid can have antitumor activity.

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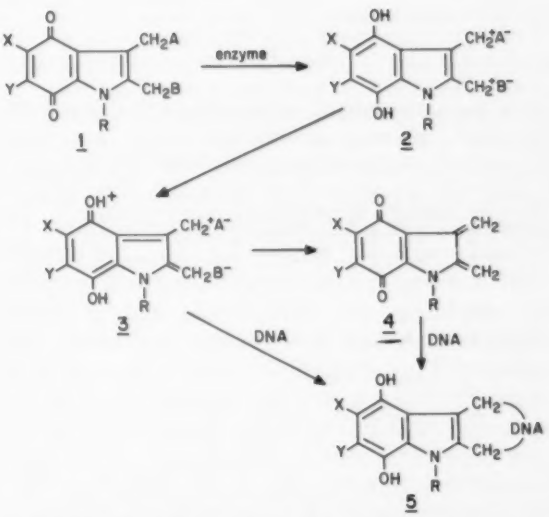
Quinones as Anticancer Agents: Potential Bioreductive Alkylating Agents

Ai Jeng Lin, Lucille A. Cosby, and Alan C. Sartorelli [1,2]

Mitomycin C,[3] an antineoplastic agent and inhibitor of the synthesis of nucleic acids (1–4), has been reported to act as a bifunctional alkylating agent which adds across both strands of the DNA double helix to cause cross-linking (5). Metabolic reduction of the benzoquinone ring of the mitomycins to the dihydro derivative is essential for both alkylation and biologic activity (6). An NADPH-dependent quinone reductase system was found to be involved in the reductive activation step (7,8). The portions of the mitomycin molecule essential for biologic activity were proposed (9,10) to be the structures shown in formulas *1* and *2*. It is envisioned that charge delocalization of the dihydroquinone hydroxyl groups of formula *2* results in the formation of the *o*-quinone methide-like (11) intermediates *3* and *4*, which are the forms that alkylate DNA.

The similarities in structure and possible chemical reactivity between the presumed active form (*4*) of the mitomycin analogs and the quinone methides, suggested that bis(*o*-quinone methides) (*6*), generated in vivo, would have a similar potential to alkylate DNA as well as other cellular structures and thereby perhaps be effective tumor-inhibitory agents. Based upon this concept, a series of benzoquinones (*8*) and naphthoquinones (*7*) with the potential to alkylate after reduction were prepared as potential analogs of mitomycin C.

The naphthoquinone derivatives (*7*) of this series were found to produce significant prolongation of the survival time of mice bearing either Adenocarcinoma 755 or Sarcoma 180 ascites cells, with considerably greater potency being exhibited in the Adenocarcinoma 755 test system (12). In general, however, benzoquinone derivatives (*8*) were only active against



Adenocarcinoma 755 (13,14). With few exceptions, the differences in maximal activity between compounds in a given tumor system were relatively small. Derivatives with two groups capable of alkylation after bioreduction appeared to be equal in cytotoxic activity to agents possessing only one arm with alkylating potential (12,13). Furthermore, similarities were observed in the antineoplastic potencies of chloromethyl, bromomethyl, and acetoxymethyl derivatives of naphthoquinones, implying that the type of leaving group present in the molecule is not critical for tumor-inhibitory activity (12). Compounds that possessed the quinone ring system, without a side chain capable of alkylation or vice versa, were inactive as tumor-inhibitory agents, suggesting that both the quinone ring and the alkylating side chain are essential for antineoplastic activity (12,13).

The mechanism of action for this class of compounds has been hypothesized (13) to involve bioreduction in vivo, in a manner analo-

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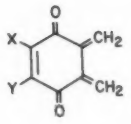
[3] Mitomycin C: NSC-26980; CAS reg. No. 50-07-7.



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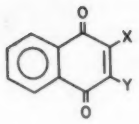
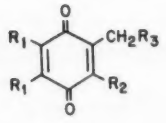


7, X, Y = −H, −CH_3, −CH_2Cl, −CH_2Br, or −CH_2OAc



8 R_1 = −CH_3, −OCH_3, or −H

R_2 = −H, −OCH_3, −CH_2OAc, −CH_2Cl or −$CH_2OC(O)NHCH_2CH_3$

R_3 = −OH, −OAc, −Cl or −$OC(O)NHCH_2CH_3$

gous to mitomycin C, by an NADPH-dependent quinone reductase enzyme system to form corresponding dihydroquinones. The dihydroquinones are unstable and spontaneously decompose to form the reactive intermediates, *o*-quinone methides. Such reactive species might well function as inhibitors of neoplastic growth by alkylation of DNA, RNA, and/or other biologic materials in a manner similar to the mitomycins. Although no evidence is currently available to support the existence of an *o*-quinone methide in vivo, chemical evidence has been obtained to substantiate the formation of this intermediate in the reductive ($NaBH_4$) amination of 2,3-dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone by aniline and morpholine (15). Should quinone methide be generated enzymatically in target cells, a similar capacity to alkylate biologic molecules is envisioned.

Inhibition of the coenzyme Q-mediated enzyme systems NADH-oxidase and succinoxidase from beef heart mitochondria has been produced by this class of compounds (12,16). Both enzyme systems are more susceptible to inhibition by benzoquinone derivatives than by the corresponding naphthoquinones, and compounds containing a long lipoidal side chain are both inactive tumor inhibitors and weaker inhibitors of coenzyme Q-mediated enzyme systems than the corresponding derivatives without an alkyl chain (16). The greater potency of the benzoquinones as inhibitors of coenzyme Q-mediated enzyme systems does not correspond to their lesser activity when compared to naphthoquinone derivatives as antineoplastic agents, suggesting that the susceptibility of mitochondrial electron transport is not the major or sole determinant in the biochemical mechanism of action of this class of compounds. Accordingly, other potential metabolic sites of action have been sought. 2,3-Bis(chloromethyl)-1,4-naphthoquinone (CAS reg. No. 31581-11-0), a member of this serieswhich is a relatively potent inhibitor of the growth of both Adenocarcinoma 755 and Sarcoma 180, was found to cause greater inhibition of the synthesis of DNA in vivo than of the formation of either RNA or protein. For example, at a dose level of 30 mg/kg, this compound produced 80% inhibition of the incorporation of thymidine-H^3 into DNA which persisted for up to 24 hours after drug treatment. Radioactivity from C^{14}-labeled 2,3-bis(chloromethyl)-1,4-naphthoquinone was found to bind tightly to DNA, RNA, and protein isolated from Sarcoma 180 ascites cells exposed to this agent, suggesting a possible alkylation of these cellular macromolecules.[4] These results encourage the further development of bioreductive alkylating agents of this type as potential clinically useful drugs.

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Early Clinical Studies With Lapachol (NSC-11905) [1]

Jerome B. Block,[2,3] Arthur A. Serpick,[2] Walter Miller,[2] and Peter H. Wiernik [2,4,5]

Lapachol has been shown to have antimalarial activity in animals (1) and is a known uncoupler of oxidative phosphorylation (2). More recently, the drug was found to have antitumor activity against Walker 256 (W256) rat carcinosarcoma but not against L1210 leukemia or Adenocarcinoma 755 in mice (3).[6] Because of this murine screening data, which seemed to have a pattern similar to other drugs clinically active against myelocytic leukemia, we began a phase I clinical trial of lapachol in 1967.

METHODS

Nineteen patients with advanced nonleukemic tumors received 21 separate courses of lapachol. In addition, two patients with chronic myelocytic leukemia in relapse were also treated. All patients had previously received a variety of therapies which had failed. During the initial course of therapy the drug was given orally in 0.25- or 0.5-g gelatin capsules. Subsequently, an oral formulation of 4 g/15 ml of syrup was used.

Either 5- or 21-day courses of the drug were used. Standard hematologic, renal, and hepatic clinical laboratory measurements were made in the course of the study. In addition, more complex tests of clotting function (prothrombin time, serum haptoglobin, silicone clotting time, fibrinogen, thromboplastin generation time, prothrombin consumption, and recalcification time) were performed in most patients treated at the higher doses of lapachol. Serum levels of lapachol were also done by a fluorimetric technique (Nayak PK, unpublished observations).

RESULTS

Dosages

Lapachol doses ranged from 250 to 3750 mg/day for 5 days. The largest total dose received was 3000 mg/day for 21 days.

Clinical Toxicity and Therapeutic Response

No toxicity was seen at doses below 1.5 g. Above this level, nausea and vomiting were usual; however, it was not clear whether this was a pharmacologic effect of lapachol or resulted from the unpalatable nature of the drug formulation. No hematologic or renal activity was seen at any dosage, although a pink color in the urine ascribed to lapachol was seen within 2 days of starting therapy. Hemolysis was not seen nor were abnormalities noted when the usual liver function tests were performed. However, at doses above 2 g/day, the prothrombin time was often markedly prolonged beyond baseline values and required correction with parenteral vitamin K. All prolongations of the prothrombin time could be corrected by this technique or by stopping the drug; the results of other tests for clotting abnormalities were normal.

One patient with metastatic breast cancer had resolution of a single osteoblastic hip lesion without change in numerous other bony lesions. All other patients, including those with chronic myelocytic leukemia, either remained clinically unchanged or had worsening of their malignancies.

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[1] Lapachol: CAS reg. No. 84–79–7; 1,4-naphthoquinone, 2-hydroxy-3-(3-methyl-2-butenyl)-.

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[4] We are indebted to Drs. D. Molins and P. Nayak, Charles Pfizer and Co., for their assistance.

[5] *Reprint requests to*: Jerome B. Block, MD, Professional Staff Associates, 1124 W Carson St, Torrance, Calif 90502.

[6] Carter SK, Newman JW, Rall D, et al. Clinical brochure: lapachol (NSC-11905). Prepared by the NCI, Bethesda, Md, Nov 1967.

Pharmacologic Studies

At single-day doses up to 40 mg/kg, lapachol was readily detectable in the plasma (table 1). At a divided daily dose of 50 mg/kg, higher plasma levels were achieved (table 2).

TABLE 1.—Plasma levels of lapachol after a single oral dose of 35–40 mg/kg in man

Time (hrs)	Plasma levels (μg/ml) Patient No. 1	Patient No. 2
1	None	None
2	10.8	None
4	11.5	15.0
8	14.6	26.4
12	10.8	14.9
16	10.4	13.2
24	6.3	5.6

TABLE 2.—Plasma levels of lapachol after daily oral doses of 50 mg/kg in man administered in 3 divided doses

Day	Plasma levels (μg/ml) Patient No. 1	Patient No. 2
1	0.0	0.0
	5.5	5.4
	20.1	9.1
2	25.5	10.1
	14.5	9.1
	20.0	8.2
3	31.6	9.1
	16.5	6.5
	35.0	12.7
4	20.0	10.9
	16.4	12.7
5	25.5	16.4
	18.2	14.5
	10.9	20.0
6	13.6	16.5

DISCUSSION

These studies showed that lapachol is well tolerated in man with the principal toxicity being nausea and vomiting and reversible prolongation of the prothrombin time at very high oral doses. If alternative dose formulations had been available, doses higher than 3.75 g/day may have been tolerated. Clinical studies were stopped mainly for two reasons. First, the appearance of an anticoagulant effect of lapachol, which may be related to its structural similarity to vitamin K, opened the question as to where such an effect was critical to the drug's antitumor activity. This had not been determined in the signal tumor screen system, W256 carcinosarcoma. It seemed important to determine whether antitumor activity without anticoagulant activity was present in animals and whether antitumor activity could persist with correction of prothrombin time prolongation by vitamin K if antitumor activity and anticoagulant activity occurred simultaneously. Secondly, a plasma level of 30 μg/ml or greater seemed critical (Nayak PK, unpublished observations) for W256 activity and such levels in man could only be reached, judging from the pharmacologic studies done, at oral doses associated with nausea, vomiting, and anticoagulation. A more readily absorbed lapachol derivative seemed to offer a more promising therapeutic potential. Other studies with lapachol in man [7] indicated that intestinal absorption was considerably less than that determined for the rat.

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Proceedings of the Symposium on Structure-Activity Techniques in the Treatment of Antitumor Drug Data

Drug Development Branch
Drug Research and Development
Division of Cancer Treatment
National Cancer Institute

Bethesda, Md
March 4, 1974

Foreword

Frank R. Quinn [1]

In recent years, considerable progress has been made in the application of quantitative structure-activity techniques to medicinal chemistry. A number of these techniques have been widely applied with apparent success in the design and improvement of drugs. Since its inception, the National Cancer Institute's (NCI) drug development program has had two primary missions: (*a*) the discovery of new antitumor agents, and (*b*) the improvement of agents which may possess less than optimal activity or produce undesirable side effects. In order to ascertain the contribution that quantitative structure-activity techniques might make to the NCI program, a symposium was held on March 4, 1974, sponsored by the Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, NCI. The subject of the symposium was: "Structure-Activity Techniques in the Treatment of Antitumor Data." The purpose of the symposium was threefold: (*a*) to acquaint NCI staff and contractors with currently used approaches in the correlation of the structure of drugs with antitumor activity; (*b*) to acquaint experts working in the structure-activity area with NCI objectives, resources, and interest in the design of better antitumor agents; and (*c*) to assess the problems that might be encountered in an attempt to apply any of these methods to NCI biologic and chemical data.

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Introduction and Overview

John S. Driscoll [1,2]

The introduction to this symposium on structure-activity techniques was primarily intended as an orientation for invited participants and guests who were not completely familiar with the National Cancer Institute's (NCI) antitumor Drug Research and Development (DR&D) program. The following four points were discussed: (*a*) objectives of the conference; (*b*) functions of DR&D; (*c*) NCI biologic screening data; and (*d*) some of the questions we hoped to answer by the end of this conference.

The conference objectives were:

1. The determination of the manner in which various structure-activity techniques are applicable to NCI in vivo antitumor data.
2. The exposure of the leaders in the field of structure-activity relationships to the NCI data base.

The functions of DR&D (consisting of two branches, the Drug Development Branch [the conference host] and the Drug Evaluation Branch) were summarized.

In a DR&D structure-activity study biologic activity data are obtained from NCI screening data summary sheets. A typical sheet was shown as an illustration. Six of the most crucial parameters were indicated and their interpretation was discussed.

As the final point, the conference participants were asked to consider some NCI *questions of practical concern relative to the various structure-activity techniques.* These were:

1. What are the major problems currently associated with the successful completion of a structure-activity study using a particular technique (eg, availability of parameters, computer costs, etc)?
2. Does the technique have a successful history of correlation with in vivo biologic data?
3. What measure of biologic data is usually utilized (eg, dose to produce a standard response)?
4. How many compounds would comprise an optimal data set?
5. How many man-months of effort are needed for a structure-activity study on a set of 25 compounds (eg, trisubstituted pyrimidines)?
   a. Are the structural parameters measured or can they be calculated?
   b. If they are calculated, is it a computer job or a manual job?
6. How much developmental work would be required to adapt a given technique to the correlation of antitumor data?
7. What are the unique advantages of any given technique?
8. Could combinations of techniques be used?

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Extrathermodynamic Approach to Drug Design

Yvonne C. Martin [1,2]

The extrathermodynamic method is an extension of the linear free-energy methods which are often used in organic chemistry. The assumption made is that the relative *biologic effect* of members of a series of drug analogs is due to a corresponding change in some *chemical property* or properties of the molecules. The effect of the various substituents on several simple chemical properties is thus statistically compared with the effect of these substituents on biologic potency.

As a measure of electronic effects, one may use, for example, the Hammett σ constant which is measured from pKa's of substituted benzoic acids. Hydrophobic effects are commonly derived from the effect of substituents on the logarithm of the octanol-water partition coefficient, the Hansch π value. Steric effects may be expressed as the radius or the molar refractivity of the substituent.

Multiple regression analysis is the most common statistical method used; the result is an equation which relates potency to physical properties. More recently, we have used discriminant analysis, a method in which activity is specified less precisely. For example, molecules in a given analysis may be specified as either active or inactive. The calculations determine which physical properties are predictive of activity class. Discriminant analysis is thus unique in that inactive compounds are included.

We have considered the in vitro potency of pargyline analogs versus monoamine oxidase. Multiple regression analysis of 47 analogs suggests an optimum pKa of 6.2, a slightly positive hydrophobic effect, and a tenfold increase in potency with ortho substitution of benzyl analogs. Three useful features of the extrathermodynamic approach may be illustrated with this series. First, the potency of four additional molecules was correctly predicted. Second, the choice of ortho substituents was such that a distinction between electronic and steric effects could not be made: thus, series should be well planned. Third, compounds that were much less potent than expected from the equation point up the special nature of the propargyl group.

Regression analysis of the antibacterial activity of erythromycin analogs was used to illustrate three additional features. First, a preliminary analysis suggested the synthesis of less lipophilic analogs. These compounds established the optimum log P. Second, similar equations were found when continuous and discontinuous (twofold dilution) data were used. Third, the optimum log P of a series of antibiotics with the same mode of action was within the range 1.1–2.4.

Another example is the antimalarial activity of naphthoquinones. Discriminant analysis of the data published by Fieser and co-workers in 1948 suggested that both log P and electron-acceptor properties determine activity. This analysis correctly predicted the activity or inactivity of 24 of 30 new series of analogs. The six errors were in series which were harder to reduce than any of the original data set. Discriminant analysis of the old plus the new data suggests that to be active the quinones must possess an E_{LEMO} (energy of lowest molecular orbital [Hückel method]) of approximately -0.385β and a log P greater than 5.5.

The extrathermodynamic approach may be useful at four stages of the followup of a "lead": (*a*) design of the initial series; (*b*) secondary followup (after a preliminary structure-activity relationship analysis); (*c*) termination of synthesis once the relevant optima are defined; and (*d*) finding new leads as suggested by deviations of certain analogs from expected activity.

This approach has four features which are

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desirable in drug design techniques: (*a*) it is based on parameters derived from physical organic chemical reactions, thus a complete theoretical understanding of the phenomenon at hand is not necessary; (*b*) the predictions are quantitative with confidence limits; (*c*) the calculations are relatively easy and inexpensive to perform; and (*d*) the analysis has application beyond the substituents and data set studied.

Discussion of the Extrathermodynamic Approach

John G. Topliss [1,2]

At the outset of the discussion period, we enumerated the following areas to be covered: (*a*) activity data, (*b*) parameters, (*c*) organization of study, (*d*) interpretation and pitfalls, and (*e*) summary. However, because of time limitations only items *a* and *b* were covered.

Most of the discussion centered on the problem of obtaining activity data in a form suitable for quantitative structure-activity analysis. The degree of data variability was considered, and a description of the methodology and a critical analysis of the basis for these data were provided by Dr. John M. Venditti. The meaning of T/C (treated/control) was discussed. It was noted that this measure reflects a net balance between the anticancer activity of a drug and its toxic effects, and therefore does not represent a pure measure of the anticancer properties of the drug. Thus, T/C for some active compounds may increase with increasing dose to a maximum value and then decline again at higher doses. It was pointed out that the selective action, rather than the potency, of the drug was critical. Thus, a compound which gives a maximum T/C value is more important than one which provides the lowest dose to produce a moderate T/C value. The latter measure has been used in previously published studies involving the extrathermodynamic approach to structure-activity relationships.

In the discussion of parameters (area *b*), the predominant role of π and log P was emphasized. It was suggested that a log P range be used as a preselection tool in handling the ordering of a large list of compounds for activity screening.

It was appropriate that the activity question dominated the discussion time allowed in view of the great importance of this factor. A discussion of areas *c–e* would clearly be profitable at some future time.

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Comparison of Hansch and Free-Wilson Methods for Structure-Activity Correlation

Paul N. Craig [1,2]

The basic principles and assumptions underlying the additivity method (Free-Wilson) for structure-activity correlation were discussed and compared with those of the multiple parameter method (Hansch). The additivity method makes no assumptions as to whether partition, polar, or steric factors are operating in a given series; instead, it assumes that each substituent makes a constant contribution towards the overall biologic activity of a molecule each time it appears at a specific position in the molecule. Thus, the only variable data used for the correlation are the observed biologic activities. If a sufficient excess number of analogs are available to permit a meaningful solution to the multiple equations in multiple unknowns, the statistical parameters enable one to test the validity of the original assumption. If it is found to hold, the resulting de novo substituent group constants may be used to predict activities of as yet unprepared analogs and to suggest possible relationships between various physical constants and biologic activity.

Examples from the antimalarial phenanthrene aminocarbinols were shown in which the additivity method and the Hansch method gave similar results when applied independently. Such corroboration strengthens confidence in the applicability of both methods for structure-activity correlation analysis. I have encountered compounds which were more readily analyzed by the Free-Wilson method than by the Hansch method, and vice versa. Therefore, it is advisable to consider applying both techniques whenever possible.

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Structure-Activity Techniques in the Treatment of Antitumor Drug Data

William P. Purcell [1,2]

To give guidance to the potential avenues of success in cancer chemotherapy, a combined quantitative structure-activity relationship (QSAR) approach is the best to use in interpreting the extraordinarily large data base (approximately 200,000 compounds). That is, without giving preference to one type of QSAR model, the various tools in QSAR should be brought to bear in sorting through the data. I envision the stepwise approach to this problem to be:

1. Application of Free-Wilson and/or Hansch models at two levels:
   (*a*) a large series of compounds in a somewhat massive analysis, and
   (*b*) a selected subseries of molecules (perhaps 50–100 compounds per series) for a more detailed analysis.
2. Based upon the results in (1), "fine tuning" could be recommended which would involve molecular orbital calculations and, perhaps, some experimental measurements such as partition coefficients, pKa's, dipole moments, and other physicochemical measurements to provide an opportunity for correlation between these properties and biologic activity.
3. The distillation of the various analyses to define somewhat simplified models which are statistically significant and consistent with experiment. These models should be used to help make decisions as to whether certain series should be pursued or dropped from consideration.

Philosophically, I feel that we should do the *most* with what we have at the present time rather than propose any other biologic tests, syntheses, or experimental work. We have an enormous data bank which has a retrievable capability, and QSAR could be applied to this data bank rather inexpensively.

I would like to propose that a team be assembled representing expertise in the Hansch analysis, the Free-Wilson analysis, molecular orbital calculations, cluster analysis, factor analysis, and pattern recognition to meet with an inhouse National Cancer Institute director who could call periodic meetings with the objective of approaching the problem as outlined above.

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Pattern Recognition

Bruce R. Kowalski [1,2]

Pattern recognition is a branch of artificial intelligence that provides an extremely powerful problem-solving tool in chemistry. The tool consists of a collection of computer programs performing data-processing tasks from the four main subsections of pattern recognition: display, preprocessing, supervised learning, and unsupervised learning. The primary attribute of pattern recognition is its ability to draw useful correlations between sought-for properties of objects that may be difficult or impossible to measure directly and multiple measurements made on the objects. Several successful chemical applications have been completed including spectral analysis, material quality control, material source identification, and, most recently, molecular structure-biologic activity correlations for anticancer drugs.

For structure-activity studies, the objects under study are retrospective and prospective drugs. The measurements made for each drug are any structural, chemical, or physical measurements that can be obtained and are thought to be related in some way to the property of interest. The property of interest can range from simply "active" or "inactive" classifications, as determined by biologic test systems, to numerical results reflecting the relative activities of drugs. A training set of previously tested drugs is input to the programs and the computer attempts to learn to classify drugs according to their activity directly from the measurements. If the measurements contain sufficient information, training will be possible, prospective drugs can be tested for activity in the computer, and the quantitative importance of the measurements can lead to improved drug design. Several other applications of considerably different nature are also possible.

At this symposium the basic philosophy of pattern recognition was presented along with a brief survey of the methods from the four subsections. The procedural aspects of applying pattern recognition to structure-activity studies was discussed, supported by the first application to purine and pyrimidine nucleoside derivatives tested for Adenocarcinoma 755 biologic activity. Finally, the batch and interactive pattern recognition systems currently in use at the University of Washington for a wide range of chemical, biomedical, and medicinal applications were introduced.

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Discussion of Pattern Recognition

S. H. Unger [1,2]

Pattern recognition techniques have been generally successful in discriminating single source data such as printed characters, voices, and most chemical spectra, but there does not seem to be sufficient consideration of the problem of selecting *relevant a priori* sources for multisource investigations. In an application of cluster analysis of substituent constants (a multisource problem), it was noted that the clusters depend upon the particular parameters chosen: what is "close," and therefore "similar," with one combination of parameters is usually neither close nor similar when a different choice of parameters is made.

A published report on the application of pattern recognition to screening prospective purines and pyrimidines in the Adenocarcinoma 755 (CA755) system (and the one discussed by Prof. Kowalski, see preceding summary) suffers from a poor choice of vector space in which to represent the activity data. Of the 200 compounds, 87 were judged to be "positives" giving a response $\geq 70\%$ tumor weight index [TWI] although 75% is cited in the original data source. Fifty structural features were coded for the purines and pyrimidines and the features were selected by a variance weight procedure. It was noted that the positive features (such as No. of S/No. of A, No. of C-S/No. of A, and No. of S-H; A=atoms) implied that carbons bonded to sulfur, unsaturation, and phenyl or halogen groups were most important, while carbonyls decreased activity. It was implied that, for example, more C-S bonds would increase the chance of obtaining an active compound, as would loading the system with phenyls. However, while it may be possible to lump these sets together by a general enough choice of vector space, if we examine the data we find this certainly not to be the case in the present study. Table 1 shows the numbers of compounds tested and the percent that were active ($\geq$ 75% TWI for CA755 and $\geq$ 30% increase in lifespan [ILS] for L1210 leukemia) stratified by heterocycle. It is immediately clear that the most successful heterocycles are 6-mercaptopurine, 6-thioguanine, and 6-halopurine. The pyrimidines, unsubstituted purines, hypoxanthines, adenines, xanthines, guanines, and 2,6-diaminopurines are unfruitful leads.

Further examination of the data set shows that, with the exception of a single compound (possessing a thienyl substituent), *all "sulfur" is present as a 6-S substituent in a purine.* Therefore, the choice of No. of C-S/No. of A, No. of C-S/No. of C, and No. of S-H is misleading; in fact, these parameters have absolutely no predictive potential yet they are the most important ones according to their variance weights. Therefore, the correct conclusion should be that 6-S is a particularly successful substructure, not that loading the heterocycle or sugar with sulfur will improve activity.

It is also curious that if our classification rule for CA755 (for example) were simply to place all 86 active 6-mercaptopurines, 6-thioguanines, and 6-halopurines in the active group (total, 99 compounds), we would obtain 86.9% "correct" predictions since there are only 13 active compounds that do not belong to any of these groups. In addition, we would obtain 85.2% "correct" predictions for the inactive group. The pattern recognition experiments achieved about 90% "correct" predictions.

Pattern recognition holds much promise for certain types of structure-activity relationships. It is often not possible to obtain precise ED50s, LD50s, etc, and structural parameters are also difficult to obtain. In such cases, pattern recognition may supplement the correlation of continuously varying data by regression ("the Hansch approach"). However, the tendency to expect computer algorithms to un-

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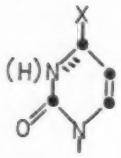
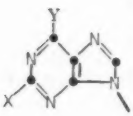
TABLE 1.—Purine and pyrimidine nucleosides active in L1210 leukemia and CA755

Group	Heterocycle	X =	Y =	L1210		CA755	
				No. of compounds tested	Active compounds* (%)	No. of compounds tested	Active compounds† (%)
Pyrimidines	Uracil	=O		44	11.4	23	8.7
	Cytosine	$-NH_2$		20	35.0	10	20.0
Purines	Purine	H	H	3	0.0	7	0.0
	Hypoxanthine	H	OH	19	5.3	14	0.0
	Adenine	H	NH_2	74	2.7	59	10.2
	6-Halopurine	H	hal	22	4.5	19	57.9
	6-Mercaptopurine	H	SR	36	50.0	52	78.8
	Xanthine	OH	OH	5	0.0	6	0.0
	Guanine	NH_2	OH	13	0.0	19	5.3
	2,6-Diaminopurine	NH_2	NH_2	3	0.0	7	28.6
	6-Thioguanine	NH_2	SR	24	66.7	38	89.5

* ≥ 30% ILS.
† ≥ 75% TWI.

cover relationships from poor data and poorer choices of vector spaces should be avoided. The problem of selecting relevant parameters (the results of regression studies may be a guide here) is quite complicated: do we accept past prejudices ("experience") or are "those who forget the past forced to repeat it?" When this problem is solved in a completely general manner, we will be able to create specific drugs at will. The problem is by no means trivial.

Use of Quantum Chemistry in the Development and Analysis of Anticancer Drugs

Ralph E. Christoffersen [1,2]

There are a variety of ways to develop effective agents for use in combating disease. At one extreme is an "Edisonian" approach, in which wholesale synthetic programs are undertaken based upon "lead compounds," in the hope that through synthesis and subsequent biologic screening more effective agents can be found. This approach has had wide-spread use in the past and has, in many instances, produced significant new drugs.

At the other extreme, the development of drugs can be viewed as a series of physicochemical events, albeit complex, each of which must be understood in order to develop optimum agents for therapeutic use. While such a "rational" program of drug design through elucidation of the complete mechanism of action cannot as yet be considered feasible at all stages, major advances have occurred both in the identification of the events involved in drug action (eg, absorption, delivery, drug-receptor interaction, metabolism, and others) and in the development of analytic tools for investigation of the various events.

One such analytic tool developed recently which has direct relevance to the understanding of one of the most important events involved in drug action, ie, drug-receptor interactions, is molecular quantum mechanics. As a result of both technical and conceptual recent advances, several quantum chemical techniques are now available for the elucidation of the geometric and electronic structural features that characterize both isolated drugs and their postulated or measured interactions with receptors. These techniques, described below, have already been applied profitably to a variety of areas of chemical and biologic interest, and they represent an important set of techniques for use in the analysis and development of more effective anticancer drugs.

The basis for the relevance of quantum chemistry as an analytic tool for use in cancer research arises from its ability, when suitably formulated and developed, to elucidate various properties of molecules for proposed mechanisms of action. These properties include the identification of conformations of molecules that are energetically accessible, the energies required to achieve them, characterization of the electronic structural features of drugs relevant to reactivity (including molecular orbital structure, charge distribution, sites of reactivity, and other properties), and investigation of the energetic requirements of proposed drug-receptor mechanisms.

Although there is still a need for much developmental work on the techniques themselves, there are several currently available techniques that can be profitably used in the study and development of anticancer drugs. These include the PCILO procedure (1,2) for rapid and reasonably reliable investigation of conformational degrees of freedom in molecules and complexes, the molecular fragment technique (3) for elucidation of electronic structural features of molecules and complexes, and others (4,5). Applications of these techniques have already occurred in a wide variety of areas of biologic interest, including characterization of energetic and electronic structural features of polypeptides (6,7) and antibiotics (8), and conformational studies on a variety of different classes of molecules, including protein residues (9), nucleosides (10), and others. Electronic and geometric features related to carcinogenesis have also been studied (11).

These techniques are also applicable to anticancer drugs and their proposed interactions with their environment, but they have not been applied to date. Hence, it is believed that by

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appropriate combination and coordination with other experimental programs, quantum chemical studies can provide valuable new insight and data for the characterization of anticancer drugs (including proposed interactions with receptors) and can contribute substantially to the development of a truly "rational" program of anticancer drug design.

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Physicochemical, Quantum Chemical, and Other Theoretical Techniques for the Understanding of the Pharmacologic Action of Drugs That Affect the Central Nervous System: Psychotropic Drugs, Narcotics, and Narcotic Antagonists and Anesthetics

Joyce J. Kaufman [1,2]

In our laboratory we have been applying quantum chemical calculations and topological, topographical, and systems analysis (supplemented by physicochemical experiments) toward the understanding of drugs that affect the central nervous system (CNS). Comparison of our accurate, non-empirical, ab initio quantum chemical calculations on medium-size heterocyclic molecules with less rigorous or semi-empirical molecular orbital calculations pointed out the differing results from these latter calculations and the care which should be taken in interpreting such results.

The overall pharmacologic effectiveness of a drug is governed by a combination of several factors. Among the major factors are the transport of the drug to the site of action and the topographical and electronic structure of the pharmacophoric portions of the molecule. The three CNS-active classes we have investigated prove to have very different pharmacologic dependencies on the combination of these two factors.

Anesthetics

Since the pioneering work of Meyer (1) and Overton (2), general anesthetic action has been observed to depend primarily on the lipid solubility of the anesthetic and to have virtually no dependence on the actual chemical structure of the molecule. We recently showed that the log of the isonarcotic pressure is proportional to the square root of a, the Van der Waal's constant which is a measure of the cohesive forces between molecules. We also derived the relationship mathematically from basic principles of thermodynamics and showed it to have this same functional form (3).

Narcotics and Narcotic Antagonists

Narcotic and narcotic antagonist actions depend both on the lipophilicities and the topographical and electronic structures of the molecules. Recent comparison of intravenous and intraventricular injections of narcotics ranging from morphine to etorphine (which is several thousand times as potent an analgesic as morphine) showed that the greater potency of etorphine was due partly to its greater lipophilicity which allowed it to cross the blood-brain barrier more easily and partly to its greater specific molecular effectiveness (4). There were only scattered data in the literature on the pKa's and partition coefficients of the narcotics. What was of even greater concern was that often only one pKa was reported for a molecule which should have had two pKa's. When we investigated we often found that these reported pKa's were neither the first nor the second but some average of the two pKa's. We set up a sensitive microtitrimetic method and mathematical deconvolution procedures which enable us to obtain accurate pKa's and partition coefficients for drugs with titratable protons (5). The oil/water distribution coefficients obtained are reported as P, which for most compounds is a much more sensitive index than log P. We have also re-emphasized that the quantity of pharmacologic importance is the drug distribution coefficient (or the "apparent" partition coefficient) rather than the "true" partition coefficient. We have also cal-

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culated the electronic structures of narcotics and narcotic antagonists by quantum chemical molecular orbital calculations and pointed out the similarities and differences between them (6). A systems analysis indicated the possible biochemical basis of analgesic action and the addictive behavior of narcotics as well as a new postulated mechanism of action for narcotic antagonists (references to the authors' earlier papers on this subject are contained in ref 6).

Psychotropic Drugs

All neuroleptics have extremely high partition coefficients, but this is not the overriding factor for their effectiveness since both the active and inactive position isomers of chlorpromazine have the same high partition coefficients. Neuroleptic drugs (the major tranquilizers) all have a common pharmacophoric topographical pattern of atoms (as distinguished from a common topological pattern of atoms) (5,7, and references to the authors' earlier work in this area contained in ref 5). This difference is a vital one because most chemical information retrieval systems are based on topological notation and thus are unsuitable data bases for searching for the proper relationships. Our quantum chemical calculations on the neuroleptic promazines and perazines and their Cl and CF_3 derivatives led us to calculate a hitherto unknown series of compounds, *N*-piperidinopromazines, and to postulate that these would be effective neuroleptics. Subsequently, a compound of this type was synthesized and is, indeed, a major tranquilizer.

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General Comments

Corwin Hansch [1,2]

This meeting can be viewed as a benchmark for a new area in structure-activity relationships (SAR). With the exception of factor analysis (eg, P. Weiner's work), we have heard discussions of all major statistical tools (long employed outside the field of chemistry) that chemists have been very slowly adopting. In addition, the use of quantum chemical approaches to SAR studies has been discussed. Anyone who scans the current literature realizes that the applications of quantum chemistry are growing at a rate almost reminiscent of the field of molecular biology. These methods are bound to become more important to the medicinal chemist. Finally, we must not overlook the structure-activity input of the traditional organic-medicinal chemistry which many of us generally take for granted. The meaningful patterns of atoms (amides, esters, heterocycles, peptides, nucleosides, etc) which have been painstakingly uncovered by years of patient study are highly significant constellations that cannot be lightly moved aside in our search for new patterns or superpatterns in SAR.

This meeting testifies to the level of maturity that cancer chemotherapy has reached. No longer are we in the position of desperately screaming for some kind, any kind, of antitumor activity. Many good leads have been uncovered and the time has come when we must, in carefully designed experiments, maximize the potency and minimize the toxicity of the lead compounds to produce the best possible drugs. Until now, it seems to me, the National Cancer Institute (NCI) has not taken as strong a role in the organization of such studies as it should.

One of the important questions raised today, and one which frequently comes up, is: Can we get meaningful quantitative SAR (QSAR) from what appear to be poor data? The answer is a relative one which hinges on how poor the data are and how much "meaning" one considers to be meaningful! In looking at hundreds of correlation equations in our laboratory we have found that standard deviations from the regression equation range from about 0.1 to 0.3. The lower value can be expected with in vitro data for relatively simple systems. With whole animal studies the standard deviation is apt to fall in the range of 0.2–0.3. Remembering that these are log units, the antilog of 0.3 is 2. If activity is expressed as log $1/C$, we can expect to predict C from our equations *within the limits studied* within a factor of $\pm$ 2 (on the average). Now, if there is a 100- or 1000-fold range in activity, this is not a bad achievement. However, if the range in activity is only fivefold or less, the QSAR are not likely to be of any value unless the biologic data are of unusual accuracy. This problem of the range of the data is so important that we have built a check into our regression program which automatically prints "POOR DATA" across the top of the page whenever there is a 0.5 or less range in a log-dependent variable. Too often we have noticed that chemists doing QSAR studies overlook this important fact. Of course, the number of data points involved and the relative noncollinearity of the independent variables also play important roles in the ultimate value of a QSAR.

For the practical problem of guidance in the exploration of a set of potential congeners, high numerical correlation between structure and activity is by no means essential, as Yvonne Martin's work with discriminant analysis shows. Setting certain limits on the probability of finding more active congeners in certain regions of substituent space or inactive ones in other regions of substituent space is far better than having no guidance at all. Even a relatively poor regression equation constitutes a

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stochastic hypothesis (1) which can be readily checked and improved by further synthesis and testing of congeners. There is nothing to lose and much to gain by using statistical techniques to follow a stochastic path to better solutions. However, we must be careful in the use of these techniques and especially in the choice of parameters; we cannot get something for nothing!

Another most important problem raised today is that of toxicity. In developing the QSAR of sets of congeners great attention is generally paid to establishing an equation for potency, and it is rare indeed that careful toxicity tests are made on the same set. In a recent study of nitrosoureas acting against L1210 leukemia in mice (2), two QSAR were formulated: one for T/*C* of 175 and one for LD10. It was interesting to note that maximum toxicity as defined by LD10 occurred at a higher log *P* than maximum T/*C*. This suggested that more hydrophilic molecules would be both more potent and less toxic if their log *P* values were in the range of –1.0–0.0. In cancer chemotherapy there is such a small differential between toxicity to host cells and tumor cells that, ideally, more specific kinds of toxicity than LD10 should be studied. It would be a most worthwhile experiment to determine careful dose-response curves under the most uniform conditions for a set of 20–25 well-chosen nitrosoureas, for which it is now known that QSAR can be formulated. At the same time, dose-response curves should be determined for the more significant kinds of toxicity. From the resulting QSARs it should be possible to attain a higher separability between toxicity to host and tumor cells. It is too much to expect that by "seat of the pants" guidance the medicinal chemist can reach the maximum separability in a variety of biologic responses which may vary in somewhat different (even nonlinear) modes with chemical structure. Until such a careful experiment is tried, we shall not know how far we can go in "rational" drug modification.

The most intractable problem, "what to do with the structures and activities of 250,000 compounds already tested by the NCI," must be considered. At present, the results from this massive effort are not available to the scientific community at large. With better computerization of these data it should be possible for the NCI to undertake exploratory projects with various specialists in the different statistical techniques discussed at this symposium. For example, if structures and activities for a few of the larger sets of interesting congeners could be supplied to the various groups who have well-developed programs for cluster analysis, regression analysis, pattern recognition, etc, it should be possible to carry out some relatively inexpensive analyses. Members of the various groups could get together to make some carefully thought out proposals for the synthesis of a few new congeners or quite different structures. Test results from these would lead to better understanding of the advantages and disadvantages of the various approaches. Such a group working together should not be considered a kind of "brain trust" to direct all synthesis, but rather a group to develop techniques for computerized SAR studies.

The widespread availability of *large* computers, placing them within reach of almost everyone, has only developed since about 1965 (advent of the IBM 360 series). Since that time we have seen a constantly accelerating number of applications of statistical and "search and compare" techniques to SAR, not to mention the swift growth of quantum chemical calculations. The next decade will see an explosive expansion in this area. The cancer chemotherapy program, to which we are nationally committed, must be in the vanguard of this extremely important development.

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A Mouse Ependymoblastoma as an Experimental Model for Screening Potential Antineoplastic Drugs [1,2,3]

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SUMMARY

The data in this paper represent the results of 2147 bioassays on 177 experimental drugs. Standard screening protocols of the National Cancer Institute's Drug Research and Development program were employed in performing these experiments. Male C57BL/6 mice weighing 20–24 g were anesthetized, intracerebrally implanted with a 1-mm^3 fragment of the ependymoblastoma tumor, and randomized into either untreated/control or drug-treated groups. Median survival time was the primary parameter measured in these studies. Untreated mice bearing the intracerebrally implanted tumor survived approximately 19.9 days. The three most active materials were: dianhydrogalactitol (NSC-132313), phenester (NSC-116785), and procarbazine (NSC-77213). Of the 14 most active materials, 12 were either alkylating agents or contained an amide-related functional group. This test system was insensitive to the ten steroids and the four drugs similar to folic acid that we studied. The ependymoblastoma tumor model was used because it was one of the four tumors recommended after a study of 16 available brain-tumor models and because it was reported to closely parallel the clinical brain-tumor situation. Results from our 106 experiments indicate that it is a consistent and reliable model. The implant technique was sufficiently rapid to make large experiments possible. We conclude that this ependymoblastoma model appears to be very useful for selecting chemotherapeutic agents with activity against this intracerebrally implanted murine tumor. Whether or not the ependymoblastoma model is predictive for selecting agents useful against human brain tumors can be judged only after parallel clinical data become available.

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Brain tumors, while accounting for only 3% of the deaths attributable to human malignant disease in the United States, pose a particularly difficult problem for neurosurgeons,

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[3] All drugs used in this study were supplied by DR&D, DCT, NCI.

[4] DR&D, DCT, NCI, Bethesda, Md.

[5] Hazleton Laboratories, Inc., Vienna, Va.

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neurologists, and clinicians because the tumor site often precludes the effective use of surgery and radiation (1). For chemotherapists, therefore, brain tumors present a rather unique challenge.

A number of experimental tumor models have been utilized in investigations directed toward improved selection of compounds with potential clinical value. An animal tumor frequently used in these studies has been the transplantable murine ependymoblastoma described by Ausman, Shapiro, and Rall (2). They identified this tumor as a mutant subline, derived from an ependymoblastoma originally induced by methylcholanthrene implantation in the mouse brain by Zimmerman and Arnold (3,4). The neural tumor, implanted intracerebrally (ic) in C57BL/6 mice, was one of four transplantable ependymoblastomas recommended as possible animal models for screening drugs for clinical use in the treatment of brain tumors by Ausman et al (2) following their review of 16 ic animal tumor models. There are many reports describing the characteristics of this and related tumors and the experimental data derived from their use as animal models (5–15).

The apparent histologic similarities between human and murine gliomas (10), the cellular stability of the ependymoblastoma, and the technical procedures permitting large-scale implantation were the major reasons for our selection of the ependymoblastoma for this study.

This report presents the preliminary evaluation of 2147 bioassays of 177 chemicals tested against the murine ependymoblastoma.

MATERIALS AND METHODS

The experimental tumor used in this research was the mutant strain derived by Ausman, Shapiro, and Rall (2) during the routine subcutaneous (sc) passage of the ependymoblastoma induced by implantation of methylcholanthrene into the brain of a mouse by Zimmerman and Arnold (3,4). Microscopic examinations[8] of hematoxylin and eosin-stained sections of the tumor during the course of this study showed the histology to be typical of an ependymoblastoma.

The tumor was propagated in C57BL/6 mice by sc implantation of a 2×2×8-mm fragment into the axillary region via a puncture in the inguinal region with a 13-gauge trocar. Fragments were routinely transplanted every 14 days.

The ic implantation was performed using a modification of the technique described by Ausman, Shapiro, and Rall (2). Fourteen-day-old sc tumor tissue was aseptically excised and placed in a sterile petri dish containing a small amount of physiologic saline. Necrotic areas were removed and the remaining tumor tissue was cut into 1-mm^3 fragments. Fragments placed at the bevel of a modified 19-gauge 3-inch Quincke-Babcock spinal needle were aspirated into the lumen of the needle by rapid, partial withdrawal of the stylet. The spinal needles were modified by having a guard placed on the outside of the barrel approximately 1.5 mm from the tip and even with the heel of the bevel. This guard limits penetration into the brain and insures uniform depth of implant.

The tip of the loaded needle was inserted through the scalp and skull at a point in the upper-right quarter of the parietal bone. This point is located on a mouse at the apex of a triangle approximately 5 mm above an imaginary straight line between the right ear and the right eye. Implantation into the right cerebral hemisphere was effected by expelling the tumor fragment with the needle's blunted stylet. Our experience has demonstrated that approximately 4%–5% of the mice receiving implants in this manner are eliminated from experimental use due to operative death, hemorrhage, or faulty implant.

The standard screening methods of the Drug Research and Development (DR&D) program were employed in this study (16). Animals utilized in these experiments were male C57BL/6 mice weighing 20–24 g and approximately 6 weeks of age. Immediately prior to tumor implant, the mice were anesthetized with the administration of a single intraperitoneal (ip) 50-mg/kg injection of hexobarbital sodium. They were randomized into untreated/control groups of 30 mice per experiment and treated

[8] Pathologic examinations were performed by R. W. Voelker, DVM, PhD, Hazleton Laboratories, Inc., Vienna, Va.

groups of six mice per dose level. A typical experiment consisted of one control group and 25 drug-treated groups. All chemicals, unless noted otherwise, were administered once daily ip on Days 1–5. Drug treatment started 24 hours after tumor implantation, which is the time specified by the DR&D protocol for the initiation of drug treatment to replace multiple drug treatments. It is recognized that this early initiation of drug treatment does not distinguish between drug effect on tumor "take" and drug effect on the course of the antineoplastic condition. The vehicle used to dissolve or suspend the compounds was either saline or hydroxypropyl cellulose. The mice were housed in suspended wire cages; food and water were available ad libitum.

The primary parameter measured in this study was the median survival time of the mice. A comparison of the test median survival time/control median survival time (%T/C) yielded a degree of increased lifespan.

Acute toxicity was evaluated on the basis of survivors on the fifth day of drug administration. Initial doses were selected on the basis of previous determinations in other rodent tumor systems.

The testing scheme employed an initial four-dose level assay with an ip injection once daily on Days 1–5. The "maximum T/C" shown in the data tables is the highest T/C for each test regimen in each experiment: in most instances this was the maximum %T/C in a multidose assay. The optimum dose shown is the dose (or range of doses) that produced the maximum %T/C in that assay. The "average maximum %T/C" is the average of the maximum %T/Cs from tests which included the optimum dose.

The following criteria for antitumor activity were employed in this study:

1. A test was considered toxic if there were $<65\%$ survivors on Day 5 or if the T/C was $\leq 85\%$. A toxic chemical was retested at lower doses until at least two nontoxic dose levels were established.
2. A test was considered inactive if the T/C was 86%–124%. An inactive chemical was retested at higher doses until a toxic dose or a 400-mg/kg injection was administered.
3. A test was considered active if the T/C was $\geq 125\%$. An active chemical was retested in an attempt to confirm the active schedule.

Selected chemicals of special clinical interest were tested to determine the optimum frequency of administration.

For quality-control purposes BCNU (NSC-409962) was included in each odd-numbered experiment.

Table 1 lists the 177 chemicals included in the 106 ependymoblastoma experiments reported in this study. Chemicals are listed in numerical order by NSC number: the name, empirical formula, and reference to pertinent data table are shown for each.

RESULTS AND DISCUSSION

The histology and pathogenesis of transplantable ependymoblastomas and other induced murine brain tumors have been described (2–4). Pathologic examination of the experimental tumor during the course of these studies showed it to be an ependymoblastoma comparable to the published descriptions. The neoplasm was well demarcated from surrounding tissue by a thin fibrous connective tissue capsule. The cells had rather scanty cytoplasm and varied from ovoid to slightly spindle-shaped. Nuclei were prominent with occasional mitotic figures. The cellular arrangement varied from nests of cells to areas where they were whorled to radiating patterns surrounding small open spaces. Occasional small areas of necrosis were present.

Our accumulated control data on untreated tumor-bearing mice are presented in table 2. These mice received no chemical treatments but they did receive injections of either saline or hydroxypropyl cellulose, whichever was used to dissolve or suspend the majority of the chemicals in the experiment. In the first 59 experiments containing 1720 control mice, the observed median survival time was 19.9 days with a standard deviation of ± 1.9 days. These results compare well with the established acceptable limits of 17–22 days (16). Of these 59 experiments, seven resulted in control median survival times outside these limits. The "no-take" tumor rate (mice alive on Day 60

after tumor implantation) was approximately 1%. Four to five percent of the mice were discarded due to faulty implant, operative death, or hemorrhage. The death distribution curve for these control mice is shown in figure 1.

Table 3 ranks 14 compounds according to the activity against the ependymoblastoma tumor; these compounds each produced an increased lifespan at least 50% longer than the controls in two separate experiments when administered by the same drug regimen (tables 4 and 5). Of these 14 compounds in table 3, ten were alkylating agents (either epoxides or mustards). One, 8-azaxanthine (NSC-756), was a purine. One, dibromodulcitol (NSC-104800), was a sugar. Seven of the 14 most effective drugs tested contained an amide-related functional group (urea, amide, imide, or lactam). Montgomery et al (17) suggested that the antitumor activity of the nitrosoureas may be due to their in situ decomposition into isocyanates. Thus, the fact that 50% of the drugs most active against the ependymoblastoma contained amide-related groups may be worthy of further consideration.

Table 6 compares the activity of three sugars. Table 7 compares the structures and activity against the ependymoblastoma of eight purines, of which only 8-azaxanthine (NSC-756) was active in our study.

The 177 chemicals tested are listed in table 1. They were received from many different suppliers and include a wide variety of chemical structures. Our results showed that 32 (18%) were active, ie, they produced an increased lifespan of at least 25% in two separate experiments using the same drug regimen. This percentage of active drugs is much higher than would be expected in a random screen of unknown drugs and it is no doubt related to the fact that most of the drugs tested in the first 106 ependymoblastoma experiments were selected because they were drugs of interest to the Division of Cancer Treatment, NCI.

Table 4 lists the six compounds most active in our study: ie, each produced an increased lifespan at least 150% longer than the controls in two separate experiments using the same drug regimen. Dianhydrogalactitol (NSC-132313) was the most active drug of the 177 studied; it is being developed toward clinical trial in the United States. Phenester (NSC-

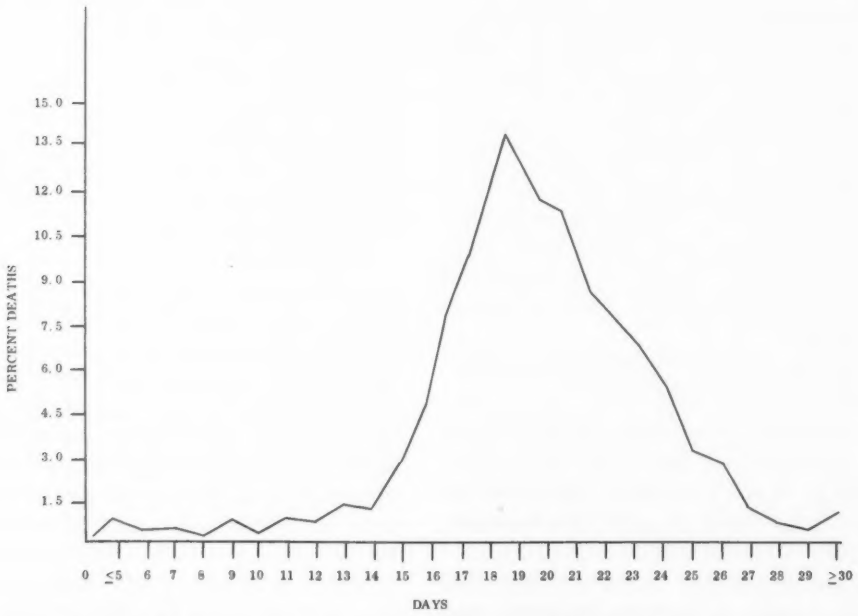


FIGURE 1.—Distribution curve of deaths of 1720 control mice in 59 ependymoblastoma experiments.

116785), the second most active drug in this study, has been dropped from development because of limiting toxicity (18). Procarbazine (NSC-77213), the third most active drug in our study, is in clinical trial. Three nitrosoureas are included in table 4 which lists the most active drugs in our study. These are CCNU (NSC-79037), the bridged CCNU (NSC-88106), and BCNU (NSC-409962).

The nitrosoureas, BCNU (NSC-409962) and CCNU (NSC-79037), both of which were very effective in prolonging the lifespan of tumor-bearing mice in our experiments, have been extensively investigated. Shapiro (7) reported that CCNU (NSC-79037) significantly retarded TdR-^{3}H uptake in murine gliomas and prolonged the lifespan of glioma-bearing mice (8,9). Shapiro, Ausman, and Rall (10) and Thompson et al (13) reported similar results with BCNU (NSC-409962). Barker et al (15) found that BCNU (NSC-409962) prolonged the lifespan of rats bearing a glioma.

Table 5 lists the materials that produced T/Cs $\geq$150% (but not 250%) in two separate ependymoblastoma experiments using the same treatment regimen.

Table 8 lists 18 compounds that showed borderline activity: ie, each produced an increased lifespan between 25% and 49% greater than the controls in two separate experiments using the same drug regimen.

Sixty-five percent of the chemicals tested were negative in our study: these 118 chemicals are listed in table 9.

Ten steroids, folic acid, and three drugs related to folic acid showed little or no activity against the ependymoblastoma as tested in this study.

Tator (11,12) demonstrated the cellular uptake of tritiated methotrexate (NSC-740) in 1 hour by ependymoblastoma tumor cells while Levin and Chadwick (5) showed that 5-fluorouracil-2-^{14}C (NSC-19893) reached a concentration in ependymoblastoma tissue in 1 hour approximately 11 times that measured in normal brain tissue. In contrast, we have found both methotrexate (NSC-740) and 5-fluorouracil (NSC-19893) to be ineffective in prolonging the life of mice with ependymoblastoma when the drugs were administered ip as a single injection on Day 1, on an intermittent schedule (every fourth day), and on a daily schedule on Days 1–5. This does not preclude the possibility that a more frequent administration of these drugs may be more advantageous.

For 27 chemicals (15% of those in the study) testing is considered to be incomplete: these are listed in table 10.

Table 11 shows the results for 13 drugs tested against the ependymoblastoma using various treatment regimens. The drugs are listed in numerical order by NSC number, and the treatment regimen employed, the optimum dose, the total dose, and the maximum %T/C observed at this dose are given. For six of the drugs, the schedule of treatment did not appear to appreciably affect the antitumor activity. Of the seven drugs in which the schedule of treatment had a significant effect, four (procarbazine [NSC-77213], CCNU [NSC-79037], methyl-CCNU [NSC-95441], and dibromodulcitol [NSC-104800]) produced the highest T/C values when they were administered on Days 1–5. Mercaptopurine riboside (NSC-4911) produced the highest T/C value (250%) when administered every 3 hours on Day 1. This was significantly higher than the rates of 110%, 125%, and 180% observed when mercaptopurine riboside (NSC-4911) was given on other regimens. 3,6-Bis(5-chloro-2-piperidyl)2,5-piperazinedione, dihydrochloride (NSC-135758) produced the highest T/C values when administered on Days 1–9 and Days 1, 5, and 9 after tumor implantation. 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) was equally effective when given as a single injection on Day 1; every fourth day, Days 1, 5, and 9; every 3 hours on Day 1; and every 3 hours on Days 1, 5, and 9 (four of the six treatment regimens utilized).

Eleven of these drugs were tested against the ependymoblastoma both on the Days 1–9 regimen and on the Days 1–5 regimen: thio-TEPA (NSC-6396), mannitol mustard (NSC-9698), 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388), cytosine arabinoside (NSC-63878), procarbazine (NSC-77213), CCNU (NSC-79037), methyl-CCNU (NSC-

95441), camptothecin (NSC-100880), dibromodulcitol (NSC-104800), 3,6-bis (5-chloro-2-piperidyl)2,5-piperazinedione, dihydrochloride (NSC-135758), and 4'-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540). Three of these were active when treatment was given on Days 1–5 but were not active when treatment was given on Days 1–9 (thioTEPA [NSC-6396], camptothecin [NSC-100880], and 4'-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside [NSC-141540]). Seven compounds active when given on Days 1–9 were also active when given on Days 1–5. One drug, cytosine arabinoside (NSC-63878), was not active on either of these regimens.

Data from the 13 drug-schedule studies are summarized in table 12. These data indicate that for six drugs the schedule of administration did not affect the activity against the tumor: these were thioTEPA (NSC-6396), mannitol mustard (NSC-9698), cytosine arabinoside (NSC-63878), camptothecin (NSC-100880), adriamycin (NSC-123127), and 4'-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540). The activity of seven of the drugs was appreciably affected by the schedule of treatment: mercaptopurine riboside (NSC-4911), 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388), procarbazine (NSC-77213), CCNU (NSC-79037), methyl-CCNU (NSC-95441), dibromodulcitol (NSC-104800), and 3,6-bis(5-chloro-2-piperidyl)2,5-piperazinedione, dihydrochloride (NSC-135758).

In summary, this preliminary evaluation of the results of antitumor bioassays of 177 selected chemicals indicates the murine ependymoblastoma to be a useful experimental tumor model for the investigation of selective chemotherapeutic effects.

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor*

NSC No.	Compound name	Empirical formula	Data table
739	Glutamic acid, *N*-[*p*[2,4-diamino-6-pteridinyl]methyl]amino)benzoyl]- **Aminopterin**	N8 O5 C19 H20	9
740	Glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]- **Methotrexate (USAN)**	N8 O5 C20 H22	9
742	Serine, diazoacetate (ester), L-	N3 O4 C5 H7	10
746	Ethyl carbamate	N O2 C3 H7	9
749	7*H*-*v*-Triazolo[4,5-*d*]pyrimidin-7-one, 5-amino-1,6-dihydro- **8-Azaguanine**	N6 O C4 H4	9
750	1,4-Butanediol, dimethanesulfonate	O6 S2 C6 H14	9
751	Butyric acid, 2-amino-4-(ethylthio), DL-	N O2 S C6 H13	10
752	Purine-6-thiol, 2-amino- **6-Thioguanine (USAN)**	N5 S C5 H5	9
755	Purine-6-thiol, monohydrate **6-Mercaptopurine**	N4 S C5 H4 · HOH	9
756	*v*-Triazolo[4,5-*d*]pyrimidine-5,7-diol **8-Azaxanthine**	N5 O2 C4 H3	5

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
762	Diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride	N Cl2 C5 H11 • HCl	9
1026	Cyclopentanecarboxylic acid, 1-amino-	N O2 C6 H11	9
1895	1,2,4-Triazolidine, 3,5-diimino- **Guanazole**	N5 C2 H5	9
3053	Actinomycin D **Dactinomycin (USAN)**	N12 O16 C62 H86	10
3054	Carbamic acid, methyl ester	N O2 C2 H5	9
3061	Pyrimidine, 2,4-diamino-5-(*p*-chlorophenyl)-6-ethyl-	N4 Cl C12 H13	9
3069	Acetamide, 2,2-dichloro-*N*-[*β*-hydroxy-*α*-(hydroxymethyl)-*p*-nitrophenethyl]- **Chloramphenicol**	N2 O5 C12 C11 H12	9
3070	4,4′-Stilbenediol, *α*,*α*′-diethyl- **Diethylstilbestrol**	O2 C18 H20	9
3073	Glutamic acid, *N*-[*p*-[[(2-amino-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]- **Folic acid**	N7 O6 C19 H19	9
3088	Butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-	N O2 Cl2 C14 H19	10
3096	Colchicine, *N*-deacetyl-*N*-methyl- **Demecolcine (USAN)** **Colcemid**	N O5 C21 H25	9
4728	1,3,4-Thiadiazole, 2-amino-	N3 S C2 H3	9
4729	1,3,4-Thiadiazole, 2-acetamido-	N3 O S C4 H5	9
4730	1,3,4-Thiadiazole, 2-(ethylamino)-	N3 S C4 H7	9
4910	9*H*-Purine, 6-chloro-9-*β*-D-ribofuranosyl-	N4 O4 Cl C10 H11	9
4911	9*H*-Purine-6-thiol, 9-*β*-D-ribofuranosyl- **Mercaptopurine riboside**	N4 O4 S C10 H12	8
5354	*o*-Phenylenediamine	N2 C6 H8	9
6396	Phosphine sulfide, tris(1-aziridinyl)- **Thio TEPA**	N3 P S C6 H12	8
8806	Alanine, 3-[*p*-[bis(2-chloroethyl)amino]-phenyl]-, monohydrochloride, L- **Melphalan (USAN)**	N2 O2 Cl2 C13 H18 • HCl	10

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
9279	*trans*-2,6-Octadien-1-ol, 3,7-dimethyl- **Geraniol**	O C10 H18	9
9698	Mannitol, 1,6-bis[(2-chloroethyl)amino]-1,6-dideoxy-, dihydrochloride, D- **Mannitol mustard**	N2 O4 Cl2 C10 H22 • 2 HCl	8
9703E	Pregn-4-ene-3,11,20-trione, 17,21-dihydroxy- **Cortisone**	O5 C21 H28	9
9706	*s*-Triazine, 2,4,6-tris(1-aziridinyl)-	N6 C9 H12	8
9717	Phosphine oxide, tris(1-aziridinyl)-	N3 O P C6 H12	8
9900E	Pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy- **Prednisolone**	O5 C21 H28	9
10018	Benzimidazole, 1-dodecyl-2-methyl-, hydrobromide	N2 H32 C20 • HBr	9
10023E	Pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy- **Prednisone (USAN)**	O5 C21 H26	10
10107	Diethylamine, 2,2'-dichloro-*N*-methyl-, *N*-oxide, compd. with HCl (1:1)	N O Cl2 C5 H11 • HCl	9
11905	1,4-Naphthoquinone, 2-hydroxy-3-(3-methyl-2-butenyl)- **Lapachol**	O3 C15 H14	9
12169E	Estra-1,3,5(10)-triene-3,16α, 17β-triol **Estriol**	O3 C18 H24	9
13875	Melamine, hexamethyl-	N6 C9 H18	9
14210	Alanine, 3-[*p*-[bis(2-chloroethyl) amino]-phenyl]-, monohydrochloride, DL- **Sarcolysin**	N2 O2 Cl2 C13 H18 • HCl	9
15200	Gallium nitrate, Ga(NO3)3	N3 O9 Ga	10
17551	1,3-Propanedione, 1-mesityl-3-(3-pyridyl)-	N O2 C17 H17	9
17663	Alanine, *N*,*N*-bis(2-chloroethyl)-, hydrochloride, DL- **Alanine mustard**	N O2 Cl2 C7 H13 • HCl	10
18429	Aniline, *N*,*N*-bis(2-chloroethyl)-	N Cl2 C10 H13	5
19893	Uracil, 5-fluoro- **5-Fluorouracil (USAN)**	N2 O2 F C4 H3	9

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
19962	3-Cyclohexene-1-carboxylic acid, 3-ethyl-4-(*p*-methoxyphenyl)-2-methyl-	O3 C17 H22	10
20534	Thioxanthen-9-one, 1-[(2-[ethyl(2-hydroxyethyl)amino]ethyl)amino]-4-methyl-, hydrochloride	N2 O2 S C20 H24 • HCl	9
23623	Acetophenone, 3',4'-dihydroxy-2-(isopropylamino)-, hydrochloride	N O3 C11 H15 • HCl	9
24117	Propionic acid, 2,2'-thiodi-, dihydrazide	N4 O2 S C6 H14	9
24559	1(2*H*)-Anthracenone, 6-[[2,6-dideoxy-3-*O*-(2,6-dideoxy-β-D-*arabino*-hexopyranosyl)-β-D-*arabino*-hexopyranosyl]oxy]-2-[(*O*-2,6-dideoxy-3-*C*-methyl-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-α-D-*lyxo*-hexopyranosyl)oxy]-(1→3)-2,6-dideoxy-β-D-*arabino*-hexopyranosyl)oxy]-3-(3,4-dihydroxy-1-methoxy-2-oxopentyl)-3,4-dihydro-8,9-dihydroxy-7-methyl- **Mithramycin (USAN)**	O24 C52 H76	9
24818	Naphtho[2,3-*d*]-1,3-dioxole-6-carboxylic acid, 5,6,7,8-tetrahydro-8-hydroxy-7-(hydroxymethyl)-5-(3,4,5-trimethoxyphenyl)-, γ-lactone **Podophyllotoxin**	O8 C22 H22	9
24901	Glycolamide, *N*,*N*'-ethylenebis-, dimethanesulfonate	N2 O8 S2 C8 H16	9
25154	Piperazine, 1,4-bis(3-bromopropionyl)- **Pipobroman (USAN)**	N2 O2 Br2 C10 H16	9
25242	Naphthalimide, *N*-(2-diethylaminoethyl)-, hydrochloride	N2 O2 C18 H20 • HCl	9
26271	2*H*-1,3,2-Oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate **Cyclophosphamide**	N2 O2 P C12 C7 H15 • HOH	8
26980	Azirino[2',3':3,4]pyrrolo-[1,2-*a*]indole-4,7-dione, 6-amino-1,1*a*,2,8,8*a*,8*b*-hexahydro-8-(hydroxymethyl)-8*a*-methoxy-5-methyl-, carbamate (ester) **Mitomycin C**	N4 O5 C15 H18	9
27640	Uridine, 2'-deoxy-5-fluoro- **Floxuridine (USAN)**	N2 O5 F C9 H11	9
29203	*trans*-Bicyclo[2.2.2]oct-5-ene-2,3-dicarbonitrile	N2 C10 H10	9

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
29422	9*H*-Purine-6-thiol, 2-amino-9-β-D-ribofuranosyl-	N5 O4 S C10 H13	9
31712	Orotic acid, 5-fluoro-, monohydrate	N2 O4 F C5 H3 • HOH	9
32065	Urea, hydroxy- **Hydroxyurea (USAN)**	N2 O2 C H4	10
32937	Acridan, 9-chloro-9-(*p*-dimethylaminophenyl)-10-ethyl-, dihydrochloride	N2 Cl C23 H23 • 2 HCl	9
32946	Guanidine, 1,1'-[(methylethanediylidene)-dinitrilo]di-, dihydrochloride, monohydrate	N8 C5 H12 • 2 HCl • HOH	9
33001E	Pregna-1,4-diene-3,20-dione, 9-fluoro-11β,17-dihydroxy-6α-methyl- Progesterone, 1-dehydro-9-fluoro-11β,17-dihydroxy-6α-methyl-	O4 F C22 H29	9
33669	2*H*-Benzo[*a*]quinolizine, 3-ethyl-1,3,4,6,7,11*b*-hexahydro-9,10-dimethoxy-2-[(1,2,3,4-tetrahydro-6,7-dimethoxy-1-isoquinolyl)methyl]-, dihydrochloride **Emetine, hydrochloride**	N2 O4 C29 H40 • 2 HCl	9
34391	Quinolinium, 1-ethyl-4-[3-(1-ethyl-4(1*H*)-quinolylidene)propenyl]—iodide **Cryptocyanine**	N2 C25 H25+ I-	9
34462	Uracil, 5-[bis(2-chloroethyl)amino]- **Uracil mustard (USAN)**	N3 O2 Cl2 C8 H11	5
34521E	Pregna-1,4-diene-3,20-dione, 9-fluoro-11β,17,21-trihydroxy-16α-methyl- Prednisolone, 9-fluoro-16α-methyl-	O5 F C22 H29	9
35843	Terephthalanilide, 4', 4"-di-2-imidazolin-2-yl-, dihydrochloride	N6 O2 C26 H24 • 2 HCl	8
36859	Quinolinium, 3-bromo-1-(3,4-dihydroxyphenacyl)—chloride	N O3 Br C17 H13+ Cl-	9
38721	Ethane, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-	Cl4 C14 H10	9
39069	L-Threitol, 1,4-dimethanesulfonate	O8 S2 C6 H14	9
39147	Glutarimide, 3-[2-hydroxy-2-(5-hydroxy-3,5-dimethyl-2-oxocyclohexyl)ethyl]- **Streptovitacin A**	N O5 C15 H23	10
39310	Naptho[1,2-*b*]quinolizinium, 13-methyl-, chloride	N C18 H14+ Cl-	9

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
40230	Acetanilide, 2-amino-4'-hydroxy-, hydrochloride	N2 O2 C8 H10 • HCl	9
44185	2*H*-Benzo[*a*]quinolizine, 3-ethyl-1,3,4,6,7,11*b*-hexahydro-9,10-dimethoxy-2-[(1,2,3,4-tetrahydro-6,7-dimethoxy-1-isoquinolyl)methyl]-, compd. with Bi(III)I3 Emetine, compd. with Bi(III)I3	N2 O4 C29 H40 • Bi(III)I3	9
45383	Picolinic acid, 5-amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolyl)-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl- **Streptonigrin (USAN)**	N4 O8 C25 H22	10
45388	Imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)- **Dacarbazine (USAN)**	N6 O C6 H10	5
47774	Piperazine, 1,4-dihydracryloyl-, dimethanesulfonate **Piposulfan (USAN)**	N2 O8 S2 C12 H22	10
49271	2(1*H*)-Pyridone, 1,5-dihydroxy-6-methyl-	N O3 C6 H7	9
49842	Vincaleukoblastine, sulfate (1:1), monohydrate **Vinblastine, sulfate, hydrate** **Velban**	N4 O9 C46 H58 • H2 SO4 • HOH	9
50741	β-D-Ribofuranoside, methyl-5-(2-chloroethylamino)-5-deoxy-2,3-*O*-isopropylidene-, hydrochloride	N O4 Cl C11 H20 • HCl	9
50857	1-Aziridineethanol, α-vinyl-, acetate, DL- **α-Vinyl AE acetate**	N O2 C8 H13	10
53396	Actinogan	Unknown	9
53398	Restrictocin	Unknown	10
56054	Pseudourea, 2-2'-(9,10-anthrylenedimethylene)bis[2-thio-, dihydrochloride, dihydrate	N4 S2 C18 H18 • 2 HCl•2 HOH	9
56408	7*H*-Pyrrolo[2,3-*d*]pyrimidine, 4-amino-7-β-D-ribofuranosyl- **Tubercidin**	N4 O4 C11 H14	9
56654	Glutamine, *N*-[1-[(1-carboxy-5-diazo-4-oxopentyl)carbamoyl]-5-diazo-4-oxopentyl]-, sodium salt **Azotomycin**	N7 O8 C17 H23 • Na salt	9

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
58404	Glycine, *N*-(diazoacetyl)-, hydrazide	N5 O2 C4 H7	10
58514	1(2*H*)-Anthracenone, 6-[[2,6-dideoxy-4-*O*-(2,6-dideoxy-4-*O*-methyl-α-D-*lyxo*-hexopyranosyl)-β-D-*lyxo*-hexopyranosyl]-oxy]-2-[(*O*-2,6-dideoxy-3-*C*-methyl-α-L-*arabino*-hexopyranosyl-(1→3)-*O*-2,6-dideoxy-β-D-*arabino*-hexopyranosyl-(1→3)-2,6-dideoxy-β-D-*arabino*-hexopyranosyl)oxy]-3-(3,4-dihydroxy-1-methoxy-2-oxopentyl)-3,4-dihydro-8,9-dihydroxy-7-methyl-, diacetate Chromomycin A3	O26 C57 H82	9
60339	Terephthalanilide, 2-chloro-4',4"-di-2-imidazolin-2-yl-	N6 O2 Cl C26 H23	10
62403	Tryptophan, 5-[bis(2-chloroethyl)amino]-, DL-	N3 O2 Cl2 C15 H19	9
62786	Benzyl alcohol, 3,4-dihydroxy-α-[(methylamino)methyl]-, (-)-	N O3 C9 H13	9
63878	Cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride **Cytarabine hydrochloride (USAN)** **Cytosine arabinoside**	N3 O5 C9 H13 • HCl	8
65346	7*H*-Pyrrolo[2,3-*d*]pyrimidine-5-carboxamide, 4-amino-7-β-D-ribofuranosyl- **Sangivamycin**	N5 O5 C12 H15	9
66020	2*H*-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-pyrrol-2-yl-	N3 O Cl C13 H10	9
66556	Acetophenone, 2-amino-3',4'-dihydroxy-, bitartrate	N O3 C8 H9 • 2 O6 C4 H6	10
67239	*as*-Triazine-3,5(2*H*,4*H*)-dione, 2-β-D-ribofuranosyl-, 2',3',5'-triacetate **Azaribine (USAN)**	N3 O9 C14 H17	9
67574	Leurocristine, sulfate (1:1) **Vincristine, sulfate (USAN)**	N4 O10 C46 H56 • H2 SO4	9
68075	Thalicarpine	N2 O8 C41 H48	8
68626	Hydrazine, 1-acetyl-2-picolinoyl-	N3 O2 C8 H9	9
69610	Purine-7-carboxylic acid, 6-(methylthio)-, ethyl ester, or 9*H*-Purine-9-carboxylic acid, 6-(methylthio)-, ethyl ester	N4 O2 S C9 H10	9

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
69945	Phosphorodiamidic acid, *N,N*-bis(2-chloroethyl)-, compd. with cyclohexylamine (1:1)	N2 O2 P Cl2 C4 H11 • N C6 H13	9
71261	9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-, monohydrate	N5 O3 S C10 H13 • HOH	9
71795	6*H*-Pyrido[4,3-*b*]carbazole, 5,11-dimethyl- **Ellipticine**	N2 C17 H14	9
74437	Piperazine, 1,4-bis(2,3-epoxypropyl)-	N2 O2 C10 H18	5
77037	**Tetrandrine (+)**	N2 O6 C38 H42	10
77213	*p*-Toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride **Procarbazine hydrochloride (USAN)**	N3 O C12 H19 • HCl	4
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso- **Lomustine (USAN)** **CCNU**	N3 O2 Cl C9 H16	4
81875	Picolinic acid, hydrazide	N3 O C6 H7	9
82151	L-*lyxo*-Hexopyranoside, 3β-acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1α-naphthacenyl-3-amino-2,3,6-trideoxy-, α-, hydrochloride **Daunomycin, hydrochloride**	N O10 C27 H29 • HCl	9
82196	Imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-	N6 O Cl2 C8 H12	9
83265	L-Alanine, 3-(tritylthio)-	N O2 S C22 H21	9
85995E	Pregn-4-ene-3,11,20-trione, 6α-fluoro-17,21-dihydroxy-, 21-acetate Corticosterone, 11-dehydro-6α-fluoro-17-hydroxy-, 21-acetate	O6 F C23 H29	9
85998	Glucopyranose, 2-deoxy-2-(3-methyl-3-nitrosoureido)-, D- **Streptozotocin**	N3 O7 C8 H15	8
88106	Urea, 1-(2-chloroethyl)-3-(2-norbornyl)-1-nitroso-	N3 O2 Cl C10 H16	4
89473	Glutamic acid, *N*-[*p*-[[2-(2-amino-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)-ethyl]amino]benzoyl]- Homofolic acid, tetrahydro-	N7 O6 C20 H25	9
91840	Isonicotinohydroxamic acid	N2 O2 C6 H6	9

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
92188	Isoquinaldaldehyde, thiosemicarbazone	N4 S C11 H10	8
94100	Mannitol, 1,6-dibromo-1,6-dideoxy-, D- **Dibromomannitol**	O4 Br2 C6 H12	8
95441	Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-, *trans*- **Semustine (USAN)** **Methyl-CCNU**	N3 O2 Cl C10 H18	5
96349	**Coralyne** Dibenzo[*a*,*g*]quinolizinium, 2,3,10,11-tetramethoxy-8-methyl-, chloride	N O4 C22 H22+ Cl– N O4 Cl C22 H22	9
100045	*Acer negundo* saponin	Unknown	9
100880	Camptothecin, sodium salt	N2 O5 C20 H18 • Na salt	8
102627	1-Propanol, 3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride	N O6 S2 C8 H19 • HCl	9
102811	Formycin	N5 O4 C10 H13	9
102816	*s*-Triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl- **5-Azacytidine**	N4 O5 C8 H12	9
104469E	Cholest-5-en, 3β-hydroxy-, [*p*-[bis(2-chloroethyl)amino]phenyl]-, acetate Cholesterol, [*p*-[bis(2-chloroethyl)amino]-phenyl]acetate	N O2 Cl2 C39 H59	9
104800	Galactitol, 1,6-dibromo-1,6-dideoxy- **Dibromodulcitol**	O4 Br2 C6 H12	5
104801	Acrylic acid, 3-*p*-anisoyl-3-bromo-, sodium salt	O4 Br C11 H9 • Na salt	10
106767	Urea, 1-(2-fluoroethyl)-1-nitroso-3-(tetrahydrothiopyran-4-yl)-, *S*,*S*-dioxide	N3 O4 S F C8 H14	8
107392	Picolinaldehyde, 5-hydroxy-, thiosemicarbazone	N4 O S C7 H8	9
109555	Carbanilide, 4,4'-diacetyl-, bis(amidinohydrazone)-, dimethanesulfonate	N10 O C19 H24 • 2 (O3 S C H4)	9
109724	1,3,2-Oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-, 2-oxide **Isophosphamide**	N2 O2 P Cl2 C7 H15	5
110435	Cesalin		10

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
112259E	Estra-1,3,5(10)-triene-3, 17β-diol, bis[[*p*-[bis(2-chloroethyl)amino]phenyl]acetate] Estradiol, diester with [*p*-[bis(2-chloroethyl)amino]phenyl]acetic acid 17β-Estradiol, bis[[*p*-[bis(2-chloroethyl)-amino]phenyl]acetate]	N2 O4 Cl4 C42 H50	9
113233	Mitomalcin (USAN)	Unknown	9
113423	Ethanesulfonic acid, compd. with 4-[*p*-(4,6-diamino-2,2-dimethyl-*s*-triazin-1-(2*H*)-yl)hydrocinnamamido]-*o*-toluenesulfonyl fluoride (1:1)	O3 S C2 H6 • N6 O3 S F C21 H25	10
114347	Quinolinium, 6-amino-4-[3-amino-4-[[*p*-(4-pyridylamino)phenyl]carbamoyl]-anilino]-1-methyl-, *p*-toluenesulfonate, metho-*p*-toluenesulfonate	N7 O C29 H29 • 2(O3 S C7 H7) N7 O7 S2 C43 H43	10
115727	Extract from *Entada phaseoloides*	Unknown	9
116785	Acetic acid, [*p*-[bis(2-chloroethyl)amino]-phenyl]-, ethyl ester **Phenester**	N O2 Cl2 C14 H19	4
118994	Diglycolaldehyde, α-(hydroxymethyl)-α'-(6-hydroxy-9*H*-purin-9-yl)-	N4 O5 C10 H10	9
119875	Platinum, diamminedichloro-, *cis*-	N2 Cl2 Pt H6	8
120949	5'-Inosinic acid, polymers, complex with 5'-cytidylic acid polymers (1:1) **Poly IC**	(N4 O8 P C10 H13)x • (N3 O8 P C9 H14)x	9
122819	Epipodophyllotoxin, 4'-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) Furo[3',4':6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one, 5,8,8a,9-tetrahydro-9-hydroxy-5-(4-hydroxy-3,5-dimethoxyphenyl)-, 9-(4,6-*O*-2-thenylidine-β-D-glucopyranoside)	O13 S C32 H32	10
123127	L-*lyxo*-Hexopyranoside, 3β-glycoloyl-1,2,3,-4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1α-naphthacenyl-3-amino-2,3,6-trideoxy-, α-, hydrochloride **Adriamycin, hydrochloride**	N O11 C27 H29 • HCl	10
126849	2,4(1*H*,3*H*)-Pyridinedione, 1-β-D-ribofuranosyl-	N O6 C10 H13	9
129220	6*H*-Furo[2',3':4,5]oxazolo[3,2-a]pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-, monoacetate (salt) *O*2,2'-Cyclocytidine, monoacetate (salt)	N3 O4 C9 H11 • O2 C2 H4	9

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
129942	2,6-Piperazinedione, 4,4'-ethylenedi-	N4 O4 C10 H14	10
129943	2,6-Piperazinedione, 4,4'-propylenedi-, (+)-	N4 O4 C11 H16	8
132313	Galactitol, 1,2:5,6-dianhydro- **Dianhydrogalactitol**	O4 C6 H10	4
133100	2,7-(Epoxypentadeca[1,11,13]trienimino)-naphtho[2,1-*b*]furan-1,11(2*H*)-dione, 5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-, 21-acetate **Rifamycin SV**	N O12 C37 H47	9
135758	2,5-Piperazinedione, 3,6-bis-(5-chloro-2-piperidyl)-, dihydrochloride	N4 O2 Cl2 C14 H22 • 2 HCl	8
141540	Epipodophyllotoxin, 4'-demethyl-, 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) Furo[3',4':6,7]naphtho[2,3-*d*]1,3-dioxol-6(5a*H*)-one, 9-[(4,6-*O*-ethylidene-β-D-glucopyranosyl)oxy]-5,8,8a,9-tetrahydro-5-(3,4,5-trimethoxyphenyl)-, [5*R*-(5α,5aβ,8aα,9β)]-	O13 C29 H32	8
141549	Methanesulfon-*m*-anisidide, 4'-(9-acridinylamino)-, monohydrochloride	N3 O3 S C21 H19 • HCl	9
141633	Cephalotaxine, 4-methyl-2-hydroxy-2-(4-hydroxy-4-methylpentyl)butanedioate ester **Homoharringtonine**	N O9 C29 H39	9
142982	Thioxanthen-9-one, 1-[[2-(diethylamino)-ethyl]amino]-4-(hydroxymethyl)-, monomethanesulfonate (salt)	N2 O2 S C20 H24 • O3 S C H4	9
143414	2,7-(Epoxypentadedeca[1,11,13]-trienimino)naphtho[2,1-*b*]furan-1,11(2*H*)-dione, 8-[*N*-(4-benzyl-2,6-dimethyl-1-piperazinyl)formimidoyl]-5,-6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-, 21-acetate, *cis*-	N4 O12 C51 H66	9
143504	Concanavalin A	Unknown	9
143647	Propionic acid, 2-amino-3-(hydroxy-nitrosamino)-, DL- Alanosine, DL-	N3 O4 C3 H7	9
143769	Methanol extraction residue (MER) of BCG	Unknown	9
143969	Fluoren-9-one, 2,7-bis[2-(diethylamino)-ethoxy]-, dihydrochloride **Tilorone**	N2 O3 C25 H33 • 2 HCl	9

TABLE 1.—Chemicals tested against mouse ependymoblastoma tumor—Continued

NSC No.	Compound name	Empirical formula	Data table
145668	6*H*-Furo[2′,3′:4,5]oxazolo[3,2-*a*]-pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-, monohydrochloride *O*2,2′-Cyclocytidine, monohydrochloride	N3 O4 C9 H11 • HCl	9
145669	4(1*H*)-Quinazolinone, 2,3-dihydro-2-(1-naphthyl)-	N6 O2 C12 H12	9
153858	Alanine, *N*-acetyl-*N*-methyl-, 6-ester with 11-chloro-6,21-dihydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-4,24-dioxa-9,22-diazatetracyclo[19.3.1.1(10,14).0(3,5)]-hexacosa-10,12,14(26),16,18-pentaene-8-23-dione **Maytansine**	N3 O10 Cl C34 H46	10
156231	Catalase		9
156232	Invertase		9
156233	Pancreatin 5X		9
400978	Spiro[cyclopropane-1,5′-[5*H*]inden]-7′(6′*H*)-one, 2′,3′-dihydro-3′,6′-dihydroxy-2′,2′,4′,6′-tetramethyl- **Illudin M**	O3 C15 H20	9
403148	Naphtho[2,3-*d*]-1,3-dioxole-6-carboxylic acid, 5,6,7,8-tetrahydro-7-(hydroxymethyl)-5-(3,4,5-trimethoxyphenyl)-, γ-lactone Silicicolin	O7 C22 H22	9
403169	7*H*-Pyrano[2,3-*c*]acridin-7-one, 3,12-dihydro-6-methoxy-3,3,12-trimethyl- **Acronycine**	N O3 C20 H19	9
404241	Adenine, 9-β-D-arabinofuranosyl-	N5 O4 C10 H13	9
409962	Urea, 1,3-bis(2-chloroethyl)-1-nitroso- **Carmustine (USAN)** **BCNU**	N3 O2 Cl2 C5 H9	4

*Two discreet chemicals were omitted from this table.

TABLE 2.—Control median survival times for ependymoblastoma controls in experiments 1–59

Control No.	Median survival time (days)	$(x-\bar{x})$	$(x-\bar{x})^2$
1	17.0	2.9	8.41
2	18.0	1.9	3.61
3	20.0	0.1	0.01
4	21.0	1.1	1.21
5	19.0	0.9	0.81
6	19.0	0.9	0.81
7	18.0	1.9	3.61
8	19.0	0.9	0.81
9	18.5	1.4	1.96
10	21.0	1.1	1.21
11	20.0	0.1	0.01
12	18.0	1.9	3.61
13	20.0	0.1	0.01
14	19.0	0.9	0.81
15	21.0	1.1	1.21
16	22.0	2.1	4.41
17	18.5	1.4	1.96
18	20.5	0.6	0.36
19	21.0	1.1	1.21
20	23.5	3.6	12.96
21	23.0	3.1	9.61
22	18.0	1.9	3.61
23	20.0	0.1	0.01
24	22.0	2.1	4.41
25	19.0	0.9	0.81
26	19.0	0.9	0.81
27	21.0	1.1	1.21
28	22.0	2.1	4.41
29	21.0	1.1	1.21
30	19.5	0.4	0.16
31	19.0	0.9	0.81
32	21.0	1.1	1.21
33	22.0	2.1	4.41
34	18.0	1.9	3.61
35	18.0	1.9	3.61
36	20.0	0.1	0.01
37	20.0	0.1	0.01
38	19.0	0.9	0.81
39	20.0	0.1	0.01
40	20.0	0.1	0.01
41	20.0	0.1	0.01
42	24.0	4.1	16.81
43	19.0	0.9	0.81
44	20.0	0.1	0.01
45	20.0	0.1	0.01
46	Not available		6.76
47	22.5	2.6	4.41
48	22.0	2.1	0.01
49	20.0	0.1	0.01
50	19.0	0.9	0.81
51	21.0	1.1	1.21
52	13.0	6.9	47.61
53	18.0	1.9	3.61
54	23.5	3.6	12.96
55	18.5	1.4	1.96
56	23.0	3.1	9.61
57	17.0	2.9	8.41
58	19.0	0.9	0.81
59	21.5	1.6	2.56
Total	1156.5		208.12 = $\Sigma(x-\bar{x})^2$.
Average	19.9		

TABLE 2.—Control median survival times for ependymoblastoma controls in experiments 1–59 — Continued

Standard deviation $= \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}} = 1.9108.$

TABLE 3.—Fourteen drugs that produced T/C values ≥ 150% in 2 separate ependymoblastoma experiments using the same regimen (listed in order of the maximum T/C average after treatment ip qd Days 1–5 for tests in which the optimum dose was included)

NSC No.	Average maximum %T/C for treatment given ip qd Days 1–5 against ependymoblastoma implanted ic	Average maximum %T/C for treatment on another schedule against ependymoblastoma implanted ic*
132313	292	—
116785	289	—
77213	280	—
79037	271	—
95441	252	266 (q4d)
756	250	—
88106	247	—
74437	225	—
18429	215	—
104800	207	—
109724	178	—
34462	160	—
45388	153	214 (q4d)
409962	150	165 (Day 1)

*— = No T/C values were better than those for treatment qd Days 1–5.

TABLE 4.—Materials that produced T/C values ≥ 250% in 2 separate ependymoblastoma experiments using the same regimen

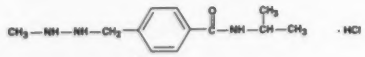
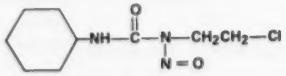
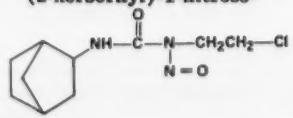
NSC No.	Compound name and structure	No. of ip treatments	Regimen	Expt No.	Dose (mg/kg/injection) Range	Dose (mg/kg/injection) Optimum	Maximum %T/C
77213	*p*-Toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride Procarbazine hydrochloride (USAN) 	1	Single dose, Day 1	11	1000–125	250	137
				46	375–165	—	All toxic
				61	768–96	384	178
				71	375–165	250	170
				77	80–55	80	266
				93	35–24	35	202
				104	15–10	10	230
		3	q4d, Days 1, 5, 9	10	400–50	100	142
				47	600–265	265	164
				61	192–12	48	247
		5	qd, Days 1–5	4	200–25	25	142
				32	75–33	33	159
				54	50–23	35	185
				72	15–10	15	152
				80	6.6–4.4	6.6	260
				102	9.9–4.3	4.3	300
		8	q3h, Day 1	61	96–12	24	185
		9	qd, Days 1–9	61	96–6.0	12	254
		24	q3h, Days 1, 5, 9	61	24–3.0	24	230
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso- Lomustine (USAN) CCNU 	1	Single dose, Day 1	62	256–16	32	139
		3	q4d, Days 1, 5, 9	62	64–4.0	32	250
		5	qd, Days 1–5	34	16–2.0	16	333
				57	24–10	10	197
				75	24–10	10	285
		8	q3h, Day 1	62	64–2.0	8.0	147
		9	qd, Days 1–9	62	64–2.0	8.0	147
		24	q3h, Days 1, 5, 9	62	8.0–0.5	1.0	154
88106	Urea, 1-(2-chloroethyl)-3-(2-norbornyl)-1-nitroso- 	5	qd, Days 1–5	41	128–16	16	300
				57	48–21	21	126
				75	48–21	32	152
				77	10–7.0	10	191
				92	4.6–3.0	4.6	250
				102	1.9–1.3	1.3	132

TABLE 4.—Materials that produced T/C values ≥ 250% in 2 separate ependymoblastoma experiments using the same regimen—Continued

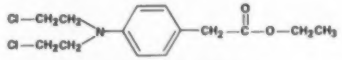
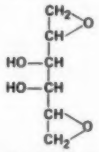
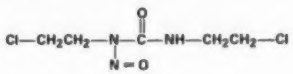
NSC No.	Compound name and structure	No. of ip treatments	Regimen	Expt No.	Dose (mg/kg/injection) Range	Dose (mg/kg/injection) Optimum	Maximum %T/C
116785	Acetic acid, [*p*-[bis(2-chloroethyl)amino]-phenyl]-, ethyl ester	5	qd, Days 1–5	44	400–50	—	All toxic
				56	35–20	—	All toxic
				70	13–8.8	13	315
				80	19–8.5	13	254
				102	8.5	8.5	300
132313	Galactitol, 1,2:5,6-dianhydro-Dianhydrogalactitol	5	qd, Days 1–5	78	16–1.0	1.0	>300
				99	6.0–0.44	0.66	>285
							
409962	Urea, 1,3-bis(2-chloroethyl)-1-nitroso-Carmustine (USAN) BCNU	1	Single dose, Day 1	1	30	—	Toxic
				2	60–15	15	141
				3	60–7.5	15	150
				5	60–7.5	30	157
				11	15	—	Toxic
				13	15	15	150
				71	15	15	129
				93	15	15	266
		3	q4d, Days 1, 5, 9	1	20–5.0	10	173
				3	20–2.5	20	150
				5	20–2.5	10	157
				9	10	—	Toxic
				47	10	—	266
		5	qd, Days 1–5	59	5.0	5.0	279
				69	5.0	5.0	292
		9	qd, Days 1–9	101	5.0	—	Toxic
		Used as positive control drug in 35 other experiments			5.0	5.0	>150

TABLE 5.—Materials that produced T/C values ≥ 150% (but not 250%) in 2 separate ependymoblastoma experiments using the same regimen

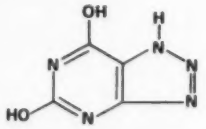
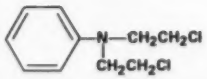
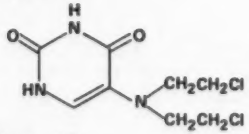
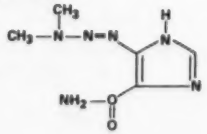
NSC No.	Compound name and structure	No. of ip treatments	Regimen	Expt No.	Dose (mg/kg/injection) Range	Dose (mg/kg/injection) Optimum	Maximum %T/C
756	v-Triazolo[4,5-*d*]pyrimidine-5,7-diol 8-Azaxanthine 	5	qd, Days 1–5	49	800–100	—	All toxic
				66	65–45	45	274
				76	65–29	65	226
				92	19–12	19	100
18429	Aniline, *N*,*N*-bis(2-chloroethyl)- 	5	qd, Days 1–5	26	200–12.5	50	147
				32	75–33	—	All toxic
				53	75–33	33	119
				72	22–5.5	5.5	100
				80	22–5.5	22	200
				102	35–14	22	300
34462	Uracil, 5-[bis(2-chloroethyl) amino]- Uracil mustard (USAN) 	5	qd, Days 1–5	34	8.0–1.0	1.0	125
				57	1.5–0.66	0.66	167
				75	1.5–0.66	1.0	190
				77	0.43–0.29	0.29	160
				92	0.19–0.12	0.19	145
45388	Imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)- Dacarbazine (USAN) 	1	Single dose, Day 1	12	600–75	75	122
				87	1000–62.5	125	272
		3	q4d, Days 1, 5, 9	9	200–25	200	137
				47	300–130	200	235
				74	450	450	204
				87	400–25	50	272
		5	qd, Days 1–5	6	100–12.5	12.5	113
				14	340–150	227	139
				32	340–150	—	All toxic
				54	100–66	66	153
		8	q3h, Day 1	87	300–18.75	18.75	272

TABLE 5.—Materials that produced T/C values ≥ 150% (but not 250%) in 2 separate ependymoblastoma experiments using the same regimen—Continued

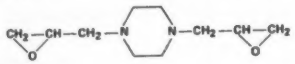
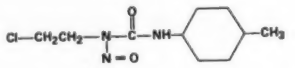
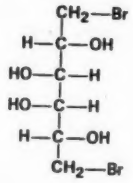
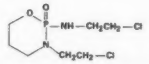
NSC No.	Compound name and structure	No. of ip treatments	Regimen	Expt No.	Dose (mg/kg/injection) Range	Dose (mg/kg/injection) Optimum	Maximum %T/C
		9	qd, Days 1–9	87	300–18.75	37.5	188
		24	q3h, Days 1, 5, 9	87	50–3.12	3.12	272
74437	Piperazine, 1,4-bis(2,3-epoxypropyl)- 	5	qd, Days 1–5	78	80–5.0	40	250
				99	60–2.2	26	200
95441	Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-, *trans*-	1	Single dose, Day 1	63	256–8.0	64	177
		3	q4d, Days 1, 5, 9	63	64–4.0	32	266
	Semustine (USAN)	5	qd, Days 1–5	34	16–2.0	16	213
	Methyl-CCNU 			59	24	—	Toxic
				69	24–10.56	10.56	292
		8	q3h, Day 1	63	64–2.0	8.0	155
		9	qd, Days 1–9	63	64–2.0	16	180
		24	q3h, Days 1, 5, 9	63	8.0–0.5	4.0	166
104800	Galactitol, 1,6-dibromo-1,6-dideoxy-	1	Single dose, Day 1	32	600–300	400	130
				64	1024–128	128	126
	Dibromodulcitol 	3	q4d, Days 1, 5, 9	10	1000–125	1000	116
				64	1024–128	256	128
		5	qd, Days 1–5	7	500–62.5	500	158
				54	600–300	400	255
				72	900	900	168
				80	1350	—	Toxic
		8	q3h, Day 1	64	1024–64	64	147
		9	qd, Days 1–9	64	1024–64	128	154
		24	q3h, Days 1, 5, 9	64	256–16	16	123
109724	1,3,2-Oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-, 2-oxide	5	qd, Days 1–5	43	200–25	100	171
				59	300	—	Toxic
				69	100–25	100	185
				80	16–11	11	163
	Isophosphamide 			100	7.2–4.8	7.2	142

TABLE 6.—Comparison of 3 sugars, showing average maximum %T/C after chronic ip administration to ependymoblastoma-bearing mice

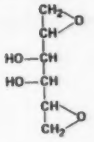
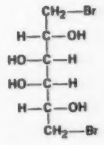
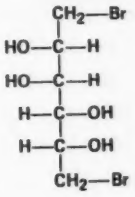
NSC No.	%T/C	Structure
132313 Galactitol, 1,2:5,6-dianhydro- Dianhydrogalactitol	>292	
104800 Galactitol, 1,6-dibromo-1,6-dideoxy- Dibromodulcitol	207	
94100 Mannitol, 1,6-dibromo-1,6-dideoxy-, D- Dibromomannitol	130	

TABLE 7.—Comparison of some purines, showing average maximum %T/C after chronic ip administration to ependymoblastoma-bearing mice

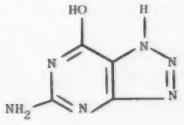
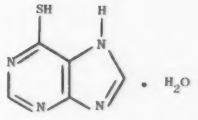
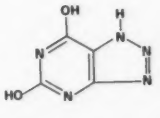
NSC No.	%T/C	Structure
749 7*H*-*v*-Triazolo[4,5-*d*]pyrimidin-7-one, 5-amino-1,6-dihydro- 8-Azaguanine	106	
755 Purine-6-thiol, monohydrate 6-Mercaptopurine	109	
756 *v*-Triazolo[4,5-*d*]pyrimidine-5,7-diol 8-Azaxanthine	250	

TABLE 7.—Comparison of some purines, showing average maximum %T/C after chronic ip administration to ependymoblastoma-bearing mice—Continued

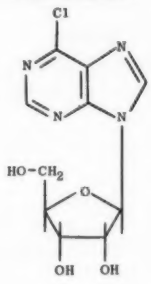
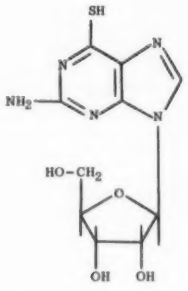
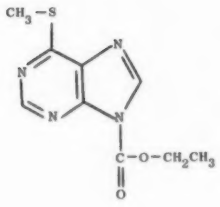
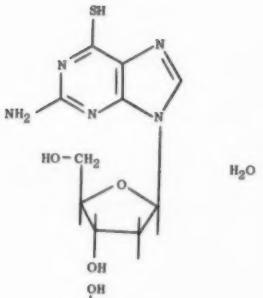
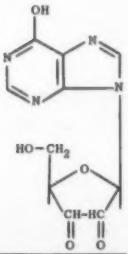
NSC No.	%T/C	Structure
4910 9*H*-Purine, 6-chloro-9-β-D-ribofuranosyl-	115	
29422 9*H*-Purine-6-thiol, 2-amino-9-β-D-ribofuranosyl-	116	
69610 Purine-7-carboxylic acid, 6-(methylthio)-, ethyl ester	108	
71261 9*H*-Purine-6-thiol, 2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-, monohydrate	110	
118994 Diglycolaldehyde, α-(hydroxymethyl)-α'-(6-hydroxy-9*H*-purin-9-yl)-	102	

TABLE 8.—Materials that produced T/C values ≥ 125% (but not 150%) in 2 separate ependymoblastoma experiments using the same regimen

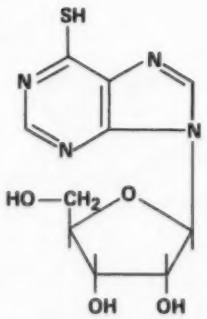
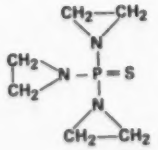
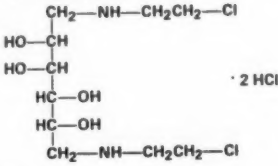
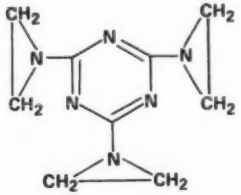
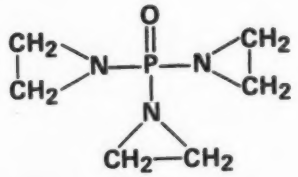
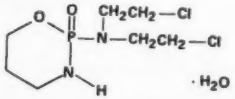
NSC No.	Compound name and structure	No. of ip treatments	Regimen	Expt No.	Dose (mg/kg/injection) Range	Dose (mg/kg/injection) Optimum	Maximum %T/C
4911	9*H*-Purine-6-thiol, 9-β-D-ribofuranosyl-Mercaptopurine riboside 	1	Single dose, Day 1	85	1000–62.5	125	110
		3	q4d, Days 1, 5, 9	85	720–60	480	125
		5	qd, Days 1–5	18	320–20	160	134
				25	240–106	240	128
				38	320–80	80	126
				52	120–55	55	180
		8	q3h, Day 1	85	240–20	160	250
		9	qd, Days 1–9	85	240–20	—	All toxic
		24	q3h, Days 1, 5, 9	85	80–5.0	—	All toxic
6396	Phosphine sulfide, tris(1-aziridinyl)-ThioTEPA 	1	Single dose, Day 1	11	24–3.0	12	127
				71	18–7.92	18	137
				81	36–2.25	9.0	126
		3	q4d, Days 1, 5, 9	8	8.0–1.0	8.0	128
				47	12.0–5.28	12	142
				81	24–1.5	6.0	138
		5	qd, Days 1–5	4	4.0–0.5	1.0	142
				32	1.5–0.66	0.66	109
				68	1.5–0.66	1.0	173
		8	q3h, Day 1	81	1.5–0.09	1.5	126
		9	qd, Days 1–9	81	1.5–0.09	1.5	124
		24	q3h, Days 1, 5, 9	81	3.0–0.18	1.5	122

TABLE 8.—Materials that produced T/C values ≥ 125% (but not 150%) in 2 separate ependymoblastoma experiments using the same regimen—Continued

NSC No.	Compound name and structure	No. of ip treatments	Regimen	Expt No.	Dose (mg/kg/injection) Range	Dose (mg/kg/injection) Optimum	Maximum %T/C
9698	Mannitol, 1,6-bis[(2-chloroethyl)amino]-1,6-dideoxy-, dihydrochloride, D-Mannitol mustard  · 2 HCl	1	Single dose, Day 1	86	180–11.2	45	127
		3	q4d, Days 1, 5, 9	86	60–3.75	15	125
		5	qd, Days 1–5	31	20–2.5	10	130
				49	15–6.6	10	132
		8	q3h, Day 1	86	20–1.25	20	140
		9	qd, Days 1–9	86	20–1.25	2.5	145
		24	q3h, Days 1, 5, 9	86	8.0–0.5	0.5	122
9706	s-Triazine, 2,4,6-tris(1-aziridinyl)- 	5	qd, Days 1–5	19	2.0–0.25	0.5	142
				27	0.75–0.33	0.5	116
				68	0.75–0.33	0.33	209
				76	0.75–0.33	0.5	109
9717	Phosphine oxide, tris(1-aziridinyl)- 	5	qd, Days 1–5	19	16–2.0	8.0	142
				27	12–5.2	12.0	119
				68	12–5.2	12.0	200
26271	2*H*-1,3,2-Oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate Cyclophosphamide 	1	Single dose, Day 1	11	1000–125	125	117
				71	62.5–15.6	31.2	129
				77	46.8–20.6	31.2	160
		3	q4d, Days 1, 5, 9	8	400–50	200	144
				74	200–50	100	175
		5	qd, Days 1–5	4	200–25	50	88
				14	12.5–3.12	12.5	123
				32	40–20	—	All toxic

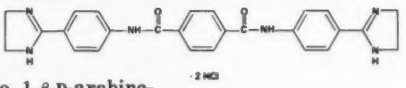
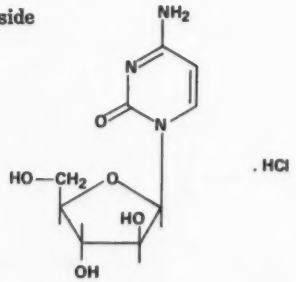
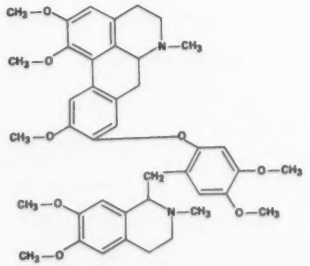
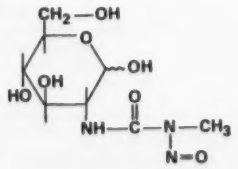
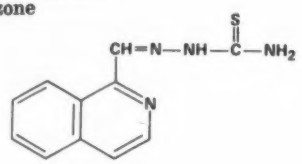
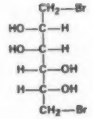
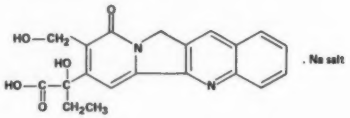
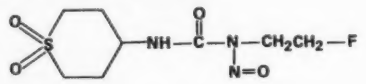
35843	Terephthalanilide, 4',4"-di-2-imidazolin-2-yl-, dihydrochloride 	5	qd, Days 1–5	34	400–50	400	130
				57	600–265	265	132
				75	600–265	400	128
63878	Cytosine, 1-β-D-arabino-furanosyl-, monohydrochloride	1	Single dose, Day 1	13	360–45	45	102
				24	818–540	818	95
				82	1000–62.5	125	114
	Cytarabine hydrochloride (USAN)	3	q4d, Days 1, 5, 9	9	120–15	15	110
				20	120–15	120	127
				47	405–180	180	133
				82	400–25	50	114
	Cytosine arabinoside 	5	qd, Days 1–5	4	60–7.5	60	121
				14	204–90	—	All toxic
		8	q3h, Day 1	82	200–12.5	12.5	93
		9	qd, Days 1–9	82	200–12.5	25	106
		24	q3h, Days 1, 5, 9	82	50–3.12	6.25	116
68075	Thalicarpine 	5	qd, Days 1–5	22	800–100	100	130
				27	150–66	150	92
				32	250–100	—	All toxic
				40	150–66	—	All toxic
				52	100–66	100	173
				54	45–29	29	106
85998	Glucopyranose, 2-deoxy-2-(3-methyl-3-nitrosoureido)-, D	5	qd, Days 1–5	41	200–25	100	127
				57	300–130	130	144
	Streptozotocin 						

TABLE 8.—Materials that produced T/C values ≥ 125% (but not 150%) in 2 separate ependymoblastoma experiments using the same regimen—Continued

NSC No.	Compound name and structure	No. of ip treatments	Regimen	Expt No.	Dose (mg/kg/injection) Range	Dose (mg/kg/injection) Optimum	Maximum %T/C
92188	Isoquinaldaldehyde, thiosemicarbazone 	5	qd, Days 1–5	43	200–25	200	118
				59	300	300	127
				80	400	400	186
94100	Mannitol, 1,6-dibromo-1,6-dideoxy-, D Dibromomannitol 	5	qd, Days 1–5	42	600–75	600	106
				55	50–35	—	All toxic
				73	23–15	23	128
				77	35–15	15	128
				91	35–15	23	121
				102	35	35	137
100880	Camptothecin, sodium salt 	1	Single dose, Day 1	89	36–2.25	36	130
		3	q4d, Days 1, 5, 9	89	12–1.5	12	110
		5	qd, Days 1–5	23	8.0–0.5	0.5	120
				28	0.25–0.12	0.12	125
				48	0.18–0.07	0.07	138
				66	1.05–0.07	0.07	122
				76	0.04	0.04	105
		8	q3h, Day 1	89	4.0–0.25	0.25	120
		9	qd, Days 1–9	89	4.0–0.25	1.0	115
		24	q3h, Days 1, 5, 9	89	1.5–0.09	0.37	115
106767	Urea, 1-(2-fluoroethyl)-1-nitroso-3-(tetrahydrothiopyran-4-yl)-, *S,S*,-dioxide 	1	Single dose, Day 1	20	64–8.0	16	127
				46	48–21	—	All toxic
		5	qd, Days 1–5	18	24–3.0	6.0	146
				32	12–6.0	—	All toxic
				52	12–1.32	6.0	434

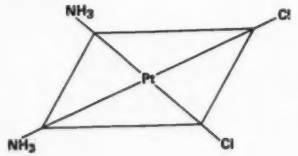
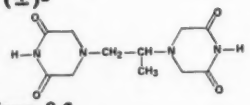
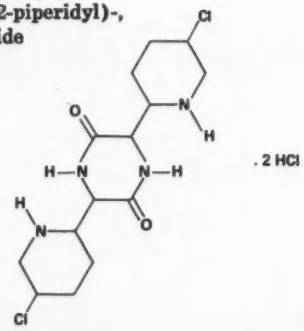
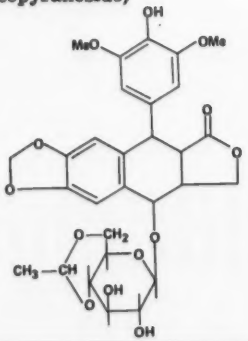
119875	Platinum, diamminedichloro-, *cis* 	5	qd, Days 1–5	34	4.0–0.5	2.0	141
				57	3.0–1.3	1.3	161
129943	2,6-Piperazinedione, 4,4′-propylenedi-, (±)- 	5	qd, Days 1–5	23	400–25	50	122
				34	12.5–6.25	12.5	130
				69	18.7–8.2	18.7	126
135758	2,5-Piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride  . 2 HCl	1	qd, Days 1–5	45	8.0–1.0	2.0	137
				56	3.0–0.44	1.32	128
				70	3.0–0.44	0.66	168
		8	q3h, Day 1	94	4.0–0.25	2.0	177
		9	qd, Days 1–9	94	4.0–0.25	0.5	272
		24	q3h, Days 1, 5, 9	94	1.5–0.09	0.75	243
141540	Epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-ethylidene-β-D-glucopyranoside) 	1	Single dose, Day 1	84	128–8.0	128	127
		3	q4d, Days 1, 5, 9	84	32–2.0	16	118
		5	qd, Days 1–5	23	64–2.0	8.0	137
				27	12–5.2	12	107
				49	32–4.0	4.0	92
				69	16–8.0	8.0	126
				76	4.0	4.0	101
				91	6.0–2.6	4.0	115
		8	q3h, Day 1	84	16–1.0	4.0	122
		9	qd, Days 1–9	84	16–1.0	4.0	120
				90	40–5.0	—	All toxic
				101	2.5–0.62	1.25	107
		24	q3h, Days 1, 5, 9	84	4.0–0.5	2.0	118

TABLE 9.—Drugs that produced T/C values < 125% when tested in the optimum dose range by ip injection qd Days 1–5

NSC No.	Dose range (mg/kg/injection)
739	0.4–0.025
740	555–6.0*
	16–2.0†
	8.0–1.0
746	1000–125
749	200–25
750	320–7.5
752	16–0.2
755	320–2.5
762	1000–0.25
1026	1000–10
1895	2270–125
3054	1135–6.25
3061	100–12.5
3069	2730–100
3070	400–50
3073	200–7.2
3096	24–3.0
4728	240–15
4729	600–50
4730	400–50
4910	2000–200
5354	680–85
9279	400–50
9703E	800–45 (sc)
9900E	335–5.5
10018	10–1.25
10107	135–5.0
11905	400–50
12169E	1200–100
13875	400–25
14210	40–0.62
17551	480–17.5
19893	108–4.0
20534	336–42
23623	680–85
24117	1110–138.75
24559	22.5–0.25
24818	16–2.0
24901	480–30
25154	200–3.12
25242	440–55
26980	8.0–0.05
27640	400–9.6
29203	8.1–0.3
29422	8.0–0.19
31712	20–0.31
32937	50–6.25
32946	200–25
33001E	160–3.8
33669	64–1.0
34391	0.8–0.1
34521E	108–8.0
36859	1045–57.5

TABLE 9.—Drugs that produced T/C values < 125% when tested in the optimum dose range by ip injection qd Days 1–5—Continued

NSC No.	Dose range (mg/kg/injection)
38721	1500–100
39069	3000–250
39310	40–0.62
40230	1080–135
44185	64–8.0
49271	440–55
49842	1.1–0.1
50741	1200–150
53396	16–0.38
56054	40–5.0
56408	4.0–0.5
56654	4.0–0.013
58514	0.8–0.02
62403	4.0–0.5
62786	8.0–0.12
65346	4.0–0.06
66020	400–22
67239	240–30
67574	2.0–0.03
68626	800–25
69610	1600–90
69945	165–6.0
71261	8.0–1.0
71795	95–8.0
81875	200–3.12
82151	4.0–0.5
82196	400–50
83265	800–25
85995E	200–0.78
89473	200–25
91840	1600–200
96349	800–100
100045	12–0.66
102627	160–20
102811	16–0.88
102816	8.0–0.19
104469E	1600–88
107392	1600–12.5
109555	320–40
112259E	800–100
113233	6.75–0.25
115727	80–4.4
118994	480–60
120949	40–5.0
126849	1200–100
129220	800–100
133100	800–100
141549	32–1.3
141633	4.0–0.25
142982	300–20
143414	800–50
143504	80–0.83
143647	400–50
143769	400–22

TABLE 9.—Drugs that produced T/C values < 125% when tested in the optimum dose range by ip injection qd Days 1–5—Continued

NSC No.	Dose range (mg/kg/injection)
143969	200–25
145668	1600–200
145669	400–50
156231	1365–100
156232	1365–100
156233	400–100
400978	2.0–0.25
403148	40–0.62‡
403169	800–12.5
404241	800–100

*Single injection, Day 1.
†Daily injections, Days 1, 5, 9.
‡Daily injections, Days 1–9.
NOTE: NSC numbers and data for discreet compounds have been eliminated from this table.

TABLE 10.—Drugs that have not yet produced T/C values ≥ 125% in two separate experiments when tested by ip injection qd Days 1–5 and drugs for which testing is incomplete

NSC No.	Dose range (mg/kg/injection)
742	32–4.0
751	335–12.5
3053	0.4–0.02
3088	48–1.0
8806	7.5–0.25
10023E	600–75* sc
15200	320–40
17663	20–0.31
19962	225–0.25
32065	800–50
39147	0.72–0.31
45383	0.08–0.01†
47774	200–25
50857	32–2.0
53398	20–0.25
58404	800–100
60339	64–3.2
66556	800–45
77037	600–50
104801	80–5.0
110435	8.0–0.002
113423	160–4.0
114347	160–2.3
122819	32–4.0*
	4.0–0.5‡
123127	8.0–0.5§
	1.6–0.1†
129942	200–12.5
153858	0.08–0.01

*Single injection, Day 1.
†q3h, Days 1, 5, 9.
‡q3h, Day 1.
§Daily injections, Days 1–9.

TABLE 11.—Results from 13 drugs tested on various regimens

NSC No.	Regimen	Optimum dose (mg/kg/injection)	Total dose (mg/kg)	Maximum %T/C
4911	Single dose, Day 1	125	125	110
	q4d, Days 1,5,9	420	1260	125
	qd, Days 1–5	55	1535	180
	q3h, Day 1	160	1280	250
6396	Single dose, Day 1	18	18	137
	q4d, Days 1, 5, 9	12	36	142
	qd, Days 1–5	1.0	5.0	173
	q3h, Day 1	1.5	12	126
	qd, Days 1–9	1.5	13.5	124
	q3h, Days 1, 5, 9	1.5	36	122
9698	Single dose, Day 1	45	45	127
	q4d, Days 1, 5, 9	15	45	125
	qd, Days 1–5	10	50	139
	q3h, Day 1	20	160	140
	qd, Days 1–9	2.9	26.1	145
	q3h, Days 1, 5, 9	0.5	12	122
45388	Single dose, Day 1	125	125	272
	q4d, Days 1, 5, 9	50	150	272
	qd, Days 1–5	66	330	153
	q3h, Day 1	18.75	150	272
	qd, Days 1–9	37.5	337.5	188
	q3h, Days 1, 5, 9	3.12	74.88	272
63878	Single dose, Day 1	125	125	114
	q4d, Days 1, 5, 9	180	540	133
	qd, Days 1–5	60	300	121
	q3h, Day 1	12.5	100	93
	qd, Days 1–9	25	225	106
	q3h, Days 1, 5, 9	6.25	150	116
77213	Single dose, Day 1	80	80	266
	q4d, Days 1, 5, 9	48	144	247
	qd, Days 1–5	4.3	21.5	300
	q3h, Day 1	24	192	185
	qd, Days 1–9	12	108	254
	q3h, Days 1, 5, 9	24	576	230
79037	Single dose, Day 1	32	32	139
	q4d, Days 1, 5, 9	32	96	250
	qd, Days 1–5	16	80	333
	q3h, Day 1	8.0	64	147
	qd, Days 1–9	8.0	72	147
	q3h, Days 1, 5, 9	1.0	24	154
95441	Single dose, Day 1	64	64	177
	q4d, Days 1, 5, 9	32	96	266
	qd, Days 1–5	10.56	52.80	292
	q3h, Day 1	8.0	64	155
	qd, Days 1–9	16	144	180
	q3h, Days 1, 5, 9	4.0	96	166
100880	Single dose, Day 1	36	36	130
	q4d, Days 1, 5, 9	12	36	110
	qd, Days 1–5	0.07	0.35	138
	q3h, Day 1	0.25	2.0	120

TABLE 11.—Results from 13 drugs tested on various regimens—Continued

NSC No.	Regimen	Optimum dose (mg/kg/injection)	Total dose (mg/kg)	Maximum %T/C
	qd, Days 1–9	1.0	9.0	115
	q3h, Days 1, 5, 9	0.37	8.8	115
104800	Single dose, Day 1	400	400	130
	q4d, Days 1, 5, 9	256	768	128
	qd, Days 1–5	400	2000	255
	q3h, Day 1	64	512	147
	qd, Days 1–9	128	1152	154
	q3h, Days 1, 5, 9	16	384	123
123127	Single dose, Day 1	8.0	8.0	106
	q4d, Days 1, 5, 9	4.0	12	120
	qd, Days 1–9	1.0	9.0	125
	q3h, Day 1	0.5	4.0	106
	q3h, Days 1, 5, 9	0.1	2.4	125
135758	qd, Days 1–5	0.66	3.3	168
	q3h, Day 1	2.0	16	177
	qd, Days 1–9	0.5	4.5	272
	q3h, Days 1, 5, 9	0.75	18	243
141540	Single dose, Day 1	128	128	127
	q4d, Days 1, 5, 9	16	48	118
	qd, Days 1–5	8.0	40	137
	q3h, Day 1	4.0	32	122
	qd, Days 1–9	4.0	36	120
	q3h, Days 1, 5, 9	2.0	48	118

TABLE 12.—Summary table of maximum %T/C values produced by 13 drugs administered on various ip schedules*

Schedule	Maximum %T/C for NSC No.—												
	4911	6396	9698	45388	63878	77213	79037	95441	100880	104800	123127	135758	141540
Single dose, Day 1	110	137	127	272	114	266	139	177	130	130	106	NT	127
q4d, Days 1, 5, 9	125	142	125	272	133	247	250	266	110	128	120	NT	118
qd, Days 1–5	180	173	139	153	121	300	333	292	138	255	NT	168	137
q3h, Day 1	250	126	140	272	93	185	147	155	120	147	106	177	122
qd, Days 1–9	NT	124	145	188	106	254	147	180	115	154	125	272	120
q3h, Days 1, 5, 9	NT	122	122	272	116	230	154	166	115	123	125	243	118

*NT = not tested.

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